European Commission

Draft Renewal Assessment Report prepared according to the Commission Regulation (EU) N° 1107/2009

Tebuconazole

1-(4-chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-ylmethyl)pentan-3-ol

Volume 3 – B.6 (AS)

Rapporteur Member State: United Kingdom
Co-Rapporteur Member State: Denmark

From March 2019
Rapporteur Member State: Denmark

Version History

When	What
March 2019	Initial draft Renewal Assessment Report (dRAR) by UK-RMS
November 2021	Initial dRAR with updated ED assessment and CLH proposal by DK-RMS
February 2023	Updated dRAR and CLH proposal by DK-RMS

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B.6. TOXICOLOGY AND METABOLISM DATA

B.6. DK-RMS	In March 2019 the UK-RMS handed over the dRAR to the new allocated DK-RMS. Before submitting the dRAR to EFSA with the purpose of public consultation DK-RMS was asked to include an assessment of the endocrine disrupting properties according to the ECHA/EFSA guidance document. In addition, DK-RMS decided a proposal for Classification and Labelling was warranted. This Vol 3 B6 section has been evaluated and written by the UK-RMS. However, The section B.6.6 Reproductive Toxicity and Section B.6.8.3 Studies on endocrine disruption have also been thoughly evaluated by DK-RMS in order to do a comprehensive ED assessment according to the ECHA/EFSA ED GD. *B.6.6 Reproductive Toxicity* DK-RMS proposes tebuconazole to be classified Repr 1B for both fertility and development, while UK-RMS concluded that Repr 2 for development is sufficient. *B.6.8.3 Studies on endocrine disruption* DK-RMS concludes that tebuconazole is an ED via the EAS-modality. The ED assessment is presented in Vol 1 section 2.10 and is based on the evaluation of the studies presented in B.6.8.3. In the two abovementioned sections DK-RMS has corrected factual errors in the study summaries as well of provided more explanation with red text. Discussions and conclusions have been provided in text boxes with blue shading like this box.

For the purpose of renewal, supplementary dossiers were received by the Bayer Task Force (comprising Bayer and Adama) and the EU Tebuconazole Task Force (comprising Helm, Sipcam Oxon, Rotam and Nufarm). The original notifiers supporting the first approval of tebuconazole were Bayer and Makhteshim. The original review of tebuconazole was conducted by Denmark. The original DAR was issued in 2006 and a relevant toxicology addendum in 2008. Following EU peer-review, an EFSA Conclusion was issued in 2008. Tebuconazole was approved as an existing active substance on 1 Sept 2009 under Inclusion Directive 2008/125/EC.1 May 2009. An updated EFSA Conclusion issued in 2014 (EFSA, 2014) is not relevant to this toxicology section as it was produced to support the amended approval of tebuconazole to include use as a plant growth regulator (in addition to the use as a fungicide).

The UK is the RMS for this renewal and Denmark is the Co-RMS. All studies previously submitted in relation to the first approval of tebuconazole have been re-evaluated to determine whether they are still valid and support the original outcome. The study summaries from these old studies from the original DAR (2006) and Addenda (2008) have been re-edited as appropriate. In particular, where new information (e.g. historical control data, additional experimental details) or new interpretation of the data has been taken into account, further details have been included. New studies and new information not previously reviewed at the EU level have been fully evaluated by the RMS.

Relevant scientifically peer-reviewed open literature publications for tebuconazole or its major metabolites identified through a literature search are discussed in this document within the relevant data point.

The end of section summaries have been fully re-drafted by the RMS and take account of information provided by both the new and previously submitted studies and the outcome of the original peer review.

Tebuconazole has harmonised classification under Regulation 1272/2008/EEC (CLP Regulation) for human health as Repr 2, H361d and Acute Tox 4 (oral), H302. Changes to the harmonised classification of tebuconazole are proposed; STOT RE 2 (eyes), REpr. 1B, Acute Tox 4, ATE 1700 mg/kg

The batches used in the toxicity tests are relevant to the original reference specification as indicated in the EFSA Conclusion 2014 (see volume 4 for further details).

B.6.1. ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION IN MAMMALS

Three kinetic studies via the oral route, owned by the Bayer Task Force, were evaluated in the original DAR (2006), peer reviewed at EU level (EFSA Conclusion, 2008), and are summarised below. In addition two new *in vitro* interspecies comparative metabolism studies, and a new protein binding study have been provided by the Bayer Task force for the purpose of renewal. Three publications of relevance to toxicokinetics have also been considered. No regulatory kinetic data have been provided by EU Tebuconazole Task Force.

B.6.1.1. Absorption, distribution, metabolism and excretion by oral route

Two oral studies investigating the absorption, distribution and excretion of [phenyl-UL-^{14}C]tebuconazole in 0.5 % aqueous tragacanth gel solution at single doses of 2 or 20 mg/kg bw, and repeat doses of 2 mg/kg bw/day for 15 days, already considered in the original DAR, are available. A third oral study investigating the metabolism of [phenyl-UL-^{14}C]tebuconazole or [triazol-3-5-^{14}C]tebuconazole at single doses of 2 or 20 mg/kg bw, and repeat doses of 2 mg/kg bw/day for 15 days, already considered in the original DAR, is also available. There are no kinetic studies of tebuconazole in animals conducted by other routes of exposure. A publication investigating metabolites of tebuconazole in human urine has been submitted by the EU Tebuconazole Task Force for the purposes of renewal.

B.6.1.1.1. *Toxicokinetics following oral exposure in the rat (single doses of either 2 mg/kg bw or 20 mg/kg bw or a repeated low dose of 2 mg/kg bw/day for 15 days)*

Previous evaluation:	In Tebuconazole DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.1.1.1/01
Study title	[Phenyl-U-^{14}C] HWG 1608: Study of biokinetic behaviour in the rat Addendum 1: [Phenyl-U-14C] HWG 1608: Study of biokinetic behaviour in the rat – Response to EPA requests and inquiries Addendum 2: [Phenyl-U-^{14}C] HWG 1608: Study of biokinetic behaviour in the rat – Raw data and additional information
Test substance	Tebuconazole (HWG 1608) uniformly labelled at the phenyl ring (phenyl-UL-^{14}C).
Purity (%) **Batch no.**	99.5 (specific activity: 84.4 µCi/mg) APF 13028500
Test animals	Male and female Wistar (BOR:WISW) rats
Groups	5 animals per group
Dose	2 and 20 mg/kg bw
Route	Oral / gavage
Vehicle	0.5% aqueous tragacanth gel solution
GLP	Yes
Guideline	OECD 417
Deviation	The following deviations from the OECD-Guideline 417 (2010) occurred: none
Acceptable	Acceptable

Methods

The absorption, distribution and excretion of tebuconazole were investigated in a GLP, OECD guideline compliant study with [phenyl-UL-^{14}C]tebuconazole in male and female Wistar rats. The test substance, suspended in a 0.5 % aqueous Tragacanth solution, was administered to male and female rats at oral doses of 2 and 20 mg/kg bw. In addition, rats of both sexes were first subjected to 14 days of treatment with a daily oral dose of 2 mg/kg of unlabelled test substance, followed by a single radioactive dose of 2 mg/kg 24 hours after the last of the doses. Furthermore, excretion of the radioactivity with the exhaled air (dose 20 mg/kg) and with the bile (dose 2 mg/kg) was studied in male rats. A summary of the nine test groups is available in Table 6.1-1.

Urine and faeces were collected from all rats at several intervals until sacrifice. Additionally, bile was collected from the bile-duct cannulated rats and plasma micro samples from the intact rats. Intact rats were sacrificed 72

hours after dosing of radiolabelled tebuconazole and blood, tissues and organs were collected. Bile-duct cannulated rats were sacrificed 48 hours after dosing of [phenyl-UL-14C]tebuconazole and GIT, skin and carcass were collected. The radioactivity was determined in all collected samples.

Table 6.1-1. Dose regimen and design of tests to investigate biokinetics in rats

Test no.	Administered dose of [phenyl-UL-^{14}C] tebuconazole	Characterisation of the experiment	Number of rats and sex	Collection of samples during the test and at sacrifice	Duration
1	20 mg/kg bw, oral	single high dose, expired air test (pilot test)	5 male	expired air, urine, faeces, GIT, skin, carcass	72 hours
2	2 mg/kg bw, oral	single low dose, bile-duct cannulation	5 male	bile, urine, faeces, GIT, skin, carcass	48 hours
3	2 mg/kg bw, oral	single low dose	5 male	urine, faeces, plasma, organs, GIT, skin, carcass	72 hours
4	2 mg/kg bw, oral	single low dose	5 female	urine, faeces, plasma, organs, GIT, skin, carcass	72 hours
5	2 mg/kg bw, oral, after 14 daily non-labelled doses at 2 mg/kg bw	multiple low dose	5 male	urine, faeces, plasma, organs, GIT, skin, carcass	72 hours
6	2 mg/kg bw, oral after 14 daily non-labelled doses at 2 mg/kg bw	multiple low dose	5 female	urine, faeces, plasma, organs, GIT, skin, carcass	72 hours
7	20 mg/kg bw, oral	single high dose	5 male	urine, faeces, plasma, organs, GIT, skin, carcass	72 hours
8	20 mg/kg bw, oral	single high dose	5 female	urine, faeces, plasma, organs, GIT, skin, carcass	72 hours
9	20 mg/kg bw, oral	single high dose, (repetition of test 7)	5 male	urine, faeces, plasma, organs, GIT, skin, carcass	72 hours

Results

Recovery

At least 92 % of the administered radioactivity was recovered in all tests (92 – 100 %), except for test 7 where 87 % of the administered radioactivity was recovered. Test 7 was repeated (as test 9) and only the results of this repeat test are presented (recovery in repeat test = 97 %). A summary of the radioactivity in percent of the administered dose found in excreta and organs and tissues at sacrifice, as well as the percentage recovery is presented in Table 6.1-2.

Table 6.1-2. Recovery of radioactivity in excreta, gastrointestinal tract and the body of rats following oral dosing of [phenyl-UL-^{14}C]tebuconazole, data presented as % of dose administered (mean of 5 animals)

Test no. **Dose** **Experiment** **Duration, sex**	**Test 1** **20 mg/kg.** **expired air test** **72 h, male**	**Test 2** **2 mg/kg.** **bile-duct cannulation** **48 h, male ***	**Test 3** **2 mg/kg** **single low dose** **72 h, male**	**Test 4** **2 mg/kg** **single low dose** **72 h, female**
Expired air	0.03	---	---	---
Urine	16.18	7.40	16.23	32.89
Bile	---	90.68	---	---
Faeces	75.81	1.50	82.11	62.48
Sum excreta	92.01	99.58	98.33	95.37
Body w/o GIT	0.44	0.21	0.54	0.34
GIT	0.33	0.01	0.25	0.35
Total body	0.77	0.22	0.79	0.69
Balance	92.80	99.80	99.12	96.06
Test no.	**Test 5**	**Test 6**	**Test 9[2]**	**Test 8**

Dose Experiment Duration, sex	2 mg/kg [1] multiple low dose[1] 72 h, male	2 mg/kg [1] multiple low dose[1] 72 h, female	20 mg/kg single high dose 72 h, male	20 mg/kg single high dose 72 h, female
Expired air	---	---	---	---
Urine	15.00	32.33	16.97	28.80
Bile	---	---	---	---
Faeces	78.77	61.46	78.73	62.73
Sum excreta	93.77	93.79	95.70	91.53
Body w/o GIT	0.67	0.42	0.63	0.24
GIT	0.41	0.96	0.39	0.30
Total body	1.08	1.38	1.02	0.54
Balance	94.85	95.17	96.72	92.07

* One of the rats died about 31 h after administration, so that the results are based on only 4 animals.
p.o. = per os, oral
[1] one radiolabelled dose after 14 daily non-labelled doses at 2 mg/kg bw
[2] repetition of test 7

Absorption
In test 2 (single low dose 2 mg/kg bw - bile-duct cannulation) the amounts of radioactivity excreted (Table 6.1-2.) with the bile (90.7 % of the administered dose) and urine (7.4 %) by the bile duct cannulated animals plus the residues in the total body at the time of sacrifice (0.23 %) showed that the radioactivity was completely absorbed after oral administration. The small amounts of radioactivity determined in the faeces of these animals might be due to fractions already absorbed and secreted and/or diffused through the gastrointestinal mucosa.

Overall, tebuconazole was completely absorbed. A figure of > 98 % of the oral dose was therefore obtained for the degree of absorption.

Toxicokinetic parameters
The analysis of the plasma curves (Table 6.1-3.) and the pharmacokinetic calculations (Table 6.1-4.) showed that the test compound was rapidly absorbed from the gastrointestinal tract (GIT) of male and female rats in all test groups indicated by t_{max} values (time after which the maximum plasma radioactivity concentration is reached) between 0.33 and 1.7 hours (calculated, Table 6.1-4). Measured t_{max} values were found between 0.33 and 3 hours.

Table 6.1-3. Time course of radioactivity in the plasma of male and female rats following an oral dose of [phenyl-UL-^{14}C]tebuconazole expressed as relative dose-normalised equivalent concentration P*

Test No. Dose Experiment Sex	Test 3 2 mg/kg single low dose male	Test 4 2 mg/kg single low dose female	Test 5 2 mg/kg [1] multiple low dose[1] male	Test 6 2 mg/kg [1] multiple low dose[1] female	Test 7 20 mg/kg, single high dose male	Test 9[2] 20 mg/kg, single high dose male	Test 8 20 mg/kg, single high dose female
0.17 h	0.0986	0.1659	0.0501	0.0781	0.0209	0.0450	0.0522
0.33 h	0.1493	**0.1974**	0.0953	0.1081	0.0779	0.1025	0.0794
0.67 h	0.1481	0.1545	0.1266	0.0973	0.0961	0.1622	**0.0941**
1.0 h	0.1455	0.1392	**0.1409**	0.1048	**0.1627**	**0.1852**	0.0902
1.5 h	**0.1504**	0.1128	0.1284	0.1120	0.1471	0.1782	0.0875
2.0 h	0.1419	---	0.1285	0.1141	0.1312	0.1562	0.0781
3.0 h	0.1295	0.0956	0.1228	**0.1170**	0.1013	0.1265	0.0680
4.0 h	0.1308	0.0908	0.1114	0.0886	0.0933	0.1131	0.0646
6.0 h	0.1190	0.0720	0.1040	0.0821	0.1070	0.1140	0.0560
8.0 h	0.1118	0.0648	0.0991	0.0306	0.0842	0.1111	0.0507
24.0 h	0.0457	0.0225	0.0416	0.0267	0.0439	0.0571	0.0182
32.0 h	0.0385	0.0169	0.0737	0.0192	0.0387	0.0595	0.0113
48.0 h	0.0231	0.0096	0.0245	0.0118	0.0274	0.0317	0.0061
56.0 h	0.0218	0.0087	0.0222	0.0109	0.0240	0.0319	0.0051

72.0 h	0.0165	0.0072	0.0155	0.0082	0.0168	0.0193	0.0044

* Relative dose normalised concentration
P percentage of residues in plasma referred to the administered dose:
P radioactivity per g plasma / administered radioactivity per g body weight (bw)
p.o. per os, oral
[1] one radiolabelled dose after 14 daily non-labelled doses at 2 mg/kg bw
[2] repetition of test 7
Bold values correspond to Pmax (respectively to a second maximum indicating an enterohepatic circulation)

Table 6.1-4. Toxicokinetic parameters after oral administration of [phenyl-UL-^{14}C]tebuconazole to male and female rats, derived from a curve analysis of dose-normalized plasma levels P

Test no. **Dose** **Experiment** **Duration, sex**	**Test 3** **2 mg/kg** **single low dose** **72 hours, male**	**Test 4** **2 mg/kg.** **single low dose** **72 hours, female**	**Test 5** **2 mg/kg [1]** **multiple low dose[1]** **72 hours, male**	**Test 6** **2 mg/kg [1]** **multiple low dose[1]** **72 hours, female**	**Test 9[2]** **20 mg/kg** **single high dose** **72 hours, male**	**Test 8** **20 mg/kg** **single high dose** **72 hours, female**
AUC experimental [h]	3.57	2.00	3.61[3]	1.96	4.24	1.52[3]
AUC total, calc. [h]	4.75	2.51	4.35[3]	2.51	5.24	1.74[4]
P_{max} (dose normalised)	0.17	0.20	0.14	0.13	0.18[3]	0.11[3]
$C_{max} = P_{max}$ x dose [µg eq/g]	0.34	0.40	0.28	0.26	3.6	2.2
t_{max} [h]	0.87	0.33	1.70	1.67	1.67[3]	1.06[4]
Terminal half-life [h]	48.46	52.46[3]	31.93	43.68	34.45[3]	34.81[4]
CL [mL/min/kg bw]	0.71	1.35	0.71	1.35	0.64[3]	1.85[3]
CL_R [mL/min/kg bw]	0.15	0.55	0.13[3]	0.54	0.13[3]	0.57[4]
MRT [h]	48.63[3]	41.89	41.55	44.27	42.73[3]	26.87[4]
Volume steady state [mL/g]	10.90	16.74	8.71	17.93	8.18[4]	14.87[3]

AUC: area under the curve. Since dose-normalized plasma levels were used for calculation all AUC values refer to the standard dose of 1 mg/kg bw. The unity is here only [h] as the concentrations (plasma level, dose level) were cancelled.
CL = clearance = administered dose x absorption / AUC
CL_R = renal clearance = fn / (AUC[0 –t_1] + AUC(t_1-t_N]) with fn = fraction of renal excretion for $t < t_N$
P: dose-normalised equivalent concentration = residue level in tissue / dose level.
P = 1 means equilibrium concentration
MRT = mean residence time
[1] one radiolabelled dose after 14 daily non-labelled doses at 2 mg/kg bw
[2] repetition of test 7
[3] : values based on 4 animals
[4]: values based on 3 animals

The measured maximum relative plasma concentrations P_{max} were achieved in the period t_{max} from 0.33 to 3 h after oral administration of 2 or 20 mg/kg p.o. (see bold values in Table 6.1-3.). Maximum measured dose-normalised plasma concentrations were in the range of 0.09 – 0.20; calculated plasma P_{max} values were in the range of 0.11 to 0.20, i.e. between only 10 and 20 % of the theoretical equilibrium concentration P = 1. Such kinetic behaviour is indicative of good tissue accessibility of the radioactivity administered with the active substance.

The values calculated for the terminal half-lives ranged from 31.9 to 52.5 hours and were, therefore, short in relation to the observation period of 72 hours. The dose-normalised areas under the plasma curves yielded a relatively wide range of AUC_{total} values (1.7 to 5.2 hours) dependent on the sex of the animals. On the basis of these relatively low values and the demonstrated 100 % absorption, correspondingly high total plasma clearances (CL) ranging from 0.6 to 1.9 mL/min were calculated.

The mean residence time of the radioactivity in the plasma ranged from 26.9 to 48.6 hours and was, therefore, short in relation to the observation period of 72 hours. The relatively high values determined for the distribution volume in steady state of 8.7 to 17.9 mL/g indicate that the radioactivity continues to be distributed unevenly in

the organism at later times after administration.

Influence of sex: A statistical analysis of the above mentioned parameters revealed sex-dependent differences between the test-groups. The males in all groups were found to have significantly larger (by a factor of 1.7 to 3) areas under the plasma curves which led to correspondingly lower total clearance values. The males treated with the high dose also exhibited a significantly longer mean residence time; the pre-treated males were found to have a significantly shorter terminal elimination half-life. These principal effects might be caused by sex-dependent metabolic differences.

Overall sex-dependent differences were apparent including generally slower clearance of tebuconazole and larger areas under plasma curves in male rats at all doses.

Influence of dose level: Analysis of the dependence of the biokinetic characteristics on the size of the dose revealed for both sexes significant differences only in those parameters describing the course of the plasma radioactivity concentration. In males, the rise in the plasma radioactivity concentration took place about 2 times more slowly after administration of the high dose. In addition, the terminal elimination of the radioactivity from the plasma was found to proceed significantly faster after the high dose. The maximal concentration in plasma was higher following administration of the high dose by a factor of 5 and 10 in males and females respectively. The females exhibited two significant effects additional to the above. After administration of the high dose, the time of achievement of the maximum radioactivity concentration in the plasma (T_{max}) was observed to increase by a factor of 3. In addition, the dose-corrected area under the plasma concentration curve (AUC) was significantly smaller in the females after the administration of the high dose.

Overall absorption of tebuconazole was slower at the high dose, with a higher peak plasma concentration and faster elimination from plasma. Additionally, corrected area under the plasma concentration curve (AUC) were significantly smaller in the females after the administration of the high dose.

Influence of pre-treatment: Pre-treatment with the unlabelled test substance led in both sexes to some differences compared to the situation after a single dose. These differences were related mainly to the characteristics derived from the course of the plasma radioactivity concentrations. Those differences, found to be statistically significant, were a slow-down in the rise of the plasma concentration in females and a shorter terminal half-life in males after pre-treatment with unlabelled test substance.

Overall, pre-treatment with the non-labelled test substance for 15 days did not lead to a significant kinetic behaviour of tebuconazole compared to that seen after single administration.

Distribution

The radioactivity remaining in the body excluding the gastrointestinal tract was very low in all test groups (Table 6.1-5.). At the end of the study, 72 h after administration, less than 1.5 % of the applied radioactivity could be detected in the organs, tissues and the remaining carcass. The mean dose-normalised relative concentrations in the animals' body were between P = 0.0027 and 0.0075 in the individual groups ("body without GIT", Table 6.1-6.). These mean dose-independent values corresponded to equivalent concentrations C = 0.0054 - 0.015 µg eq/g after administration of 2 mg/kg and to C = 0.054 - 0.150 µg eq/g after administration of a 10-times higher dose of 20 mg/kg.

Highest residues were found in the liver (P = 0.0284 – 0.0398), one of the organs responsible for metabolism and excretion of the test compound and its metabolites. Normalised liver levels were about 5 times the mean concentrations in the males and about 10 times in the females. The relative concentrations in the majority of tissues and organs were lower or higher than the mean concentrations in the animal body of each group by a factor of about 2. In the majority of the test groups the bones and the brain were among the tissues with the lowest radioactivity concentrations: about 3 times lower than the mean concentrations measured in the bodies in the individual groups.

Sex-dependent differences between the corresponding groups could be observed; the radiolabelled residues determined in all tissues and organs at the end of the study (72 h after administration) were generally low, but 1.5 - 2.5 times higher in the males of all groups than in the corresponding females.

No accumulation of tebuconazole residues was indicated in any organ or tissue after oral administration of radiolabelled tebuconazole as proven by the low levels in Tables 6.1-5 and 6.1-6.

Overall, tebuconazole was rapidly and well distributed into organs and tissues with highest levels found in the liver. Tebuconazole did not accumulate in any organ or tissue after oral administration and was rapidly excreted. Radiolabelled residues in tissues and organs were low at termination, but generally higher in male rats compared to females.

Table 6.1-5. Radioactive residues in organs and tissues 72 h after oral administration of [phenyl-UL-^{14}C]tebuconazole expressed as % of dose

Test no. **Dose** **Experiment** **Duration, sex**	**Test 3** **2 mg/kg single low dose** **72 h, male**	**Test 4** **2 mg/kg single low dose** **72 h, female**	**Test 5** **2 mg/kg [1] multiple low dose1** **72 h, male**	**Test 6** **2 mg/kg [1] multiple low dose1** **72 h, female**	**Test 9[2]** **20 mg/ single high dose** **72 h, male**	**Test 8** **20 mg/kg single high dose** **72 h, female**
Liver	0.1359	0.1496	0.1488	0.1429	0.1392	0.1084
Spleen	0.0010	0.0006	0.0015	0.0006	0.0010	0.0009
Kidney	0.0077	0.0031	0.0098	0.0042	0.0118	0.0022
Perirenal fat	0.0009	0.0007	0.0015	0.0008	0.0002	0.0008
Testis	0.0069	N/A	0.0084	N/A	0.0077	N/A
Ovaries	N/A	0.0001	N/A	0.0002	N/A	0.0005
Uterus	N/A	0.0008	N/A	0.0023	N/A	0.0002
Muscle (femur)	0.0028	0.0011	0.0034	0.0020	0.0038	0.0008
Bone (femur)	0.0007	0.0004	0.0011	0.0004	0.0008	0.0006
Skin	0.1460	0.0448	0.1046	0.0798	0.1326	0.0303
Plasma	0.0109	0.0070	0.0148	0.0088	0.0098	0.0044
Erythrocytes	0.0171	0.0054	0.0187	0.0069	0.0189	0.0028
Heart	0.0008	0.0010	0.0025	0.0012	0.0044	0.0008
Brain	0.0017	0.0011	0.0028	0.0013	0.0038	0.0007
Lung	0.0012	0.0041	0.0094	0.0056	0.0107	0.0024
Residual carcass	0.2034	0.1068	0.3392	0.1476	0.2877	0.0834
GIT	0.2719	0.3464	0.5756	0.9612	0.3927	0.3572
Body w/o GIT	0.5370	0.3266	0.6666	0.4045	0.6324	0.2391

p.o. = per os, oral; N/A = not applicable
[1] one radiolabelled dose after 14 daily non-labelled doses at 2 mg/kg bw
[2] repetition of test 7

Table 6.1-6. Radioactive residues in organs and tissues 72 h after oral administration of [phenyl-UL-^{14}C]tebuconazole expressed as dose-normalised equivalent concentration P

Test No. **Dose** **Experiment** **Duration, sex**	**Test 3** **2 mg/kg. single low dose** **72 h, male**	**Test 4** **2 mg/kg single low dose** **72 h, female**	**Test 5** **2 mg/kg [1] multiple low dose[1]** **72 h, male**	**Test 6** **2 mg/kg [1] multiple low dose[1]** **72 h, female**	**Test 9[2]** **20 mg/kg single high dose** **72 h, male**	**Test 8** **20 mg/kg single high dose** **72 h, female**
Liver	0.03300	0.03620	0.03320	0.03980	0.03050	0.02840
Spleen	0.00517	0.00243	0.00780	0.00279	0.00533	0.00293
Kidney	0.01290	0.00501	0.01130	0.00681	0.01680	0.00299
Perirenal fat	0.00511	0.00269	0.01010	0.00451	0.00152	0.00391
Testis	0.00437	N/A	0.00515	N/A	0.00439	N/A
Ovaries	N/A	0.00359	N/A	0.00530	N/A	0.01260
Uterus	N/A	0.00397	N/A	0.00653	N/A	0.00077
Muscle (femur)	0.00275	0.00127	0.00258	0.00155	0.00366	0.00089

Bone (femur)	0.00236	0.00136	0.00348	0.00127	0.00288	0.00174
Skin	0.00729	0.00257	0.00488	0.00415	0.00683	0.00139
Plasma	0.01500	0.00614	0.01470	0.00692	0.01730	0.00426
Erythrocytes	0.01080	0.00367	0.01000	0.00440	0.01530	0.00212
Heart	0.00245	0.00263	0.00600	0.00311	0.01040	0.00181
Brain	0.00208	0.00124	0.00270	0.00141	0.00448	0.00084
Lung	0.00191	0.00474	0.00973	0.00639	0.01210	0.00325
Residual carcass	0.00363	0.00186	0.00609	0.00222	0.00536	0.00137
GIT	0.02760	0.02590	0.04110	0.09920	0.03330	0.02980
Body w/o GIT	0.00605	0.00347	0.00745	0.00423	0.00718	0.00265

p.o. = per os, oral; N/A = not applicable
[1] one radiolabelled dose after 14 daily non-labelled doses at 2 mg/kg bw
[2] repetition of test 7

Excretion
The excretion behaviour was investigated by measurement of the radioactivity in the expired air, bile, urine and faeces. The residual radioactivity was determined by the analysis of the carcass and tissues after sacrifice.

The expiration of ^{14}C-carbon dioxide and other ^{14}C-labelled volatile compounds amounted only to 0.032 % of the administered dose during the 72 hours following a single oral administration of 20 mg/kg [phenyl-UL-^{14}C]tebuconazole as shown in the pilot experiment (expired air test 1; Table 6.1-7.). This demonstrates the high metabolic stability of the phenyl labelling position.

The overall excretion of the radioactivity was a fast and complete process. Within 72 h of administration (dose 2 or 20 mg/kg bw, single or pre-treatment) between 91.5 and 98.4 % of the administered radioactive dose (99 % of the recovered radioactivity) was excreted with the urine and faeces (Table 6.1-7.). The major route of excretion was the faecal route (and biliary in case of bile duct-cannulated animals). Depending on the sex of the animals, about 15 - 33 % of the administered dose was excreted with the urine and about 62 – 82 % of the dose with the faeces.

The renal excretion of male animals of all test groups was half as much as that of the females; the proportion of radioactivity excreted with the faeces (and bile) was correspondingly higher in the males. These differences were in all cases significant.

Male animals with biliary fistulae (dose 2 mg/kg) eliminated approx. 91 % of the dose with the bile within 48 h of administration, about 7.4 % with the urine, and only 1.5 % with faeces. Therefore, large quantities of faecal radioactivity of intact animals could be assigned to a biliary excretion into the intestinal lumen. Male animals with biliary fistulae excreted about half of the quantity of radioactivity with the urine within 48 hours after administration compared to corresponding intact males within 72 hours, indicating enterohepatic re-circulation of the radioactivity.

The biliary elimination of radioactivity was very fast: 50 % of the total biliary excretion was already eliminated after 2.5 h and 90 % after 7 h indicating a significant first pass effect.

The radioactivity was also found to undergo relatively rapid excretion by the renal route, 50 % of the total renal excretion occurred within 11 - 16 h of administration and 90 % within 29 - 36 h. The renal clearance (CLR) values determined in the various groups of animals by model-independent plasma curve analysis were between 0.13 and 0.57 mL/min.

Overall, excretion of the radioactivity was a fast and complete process. The major route of excretion was the faecal route (62 – 82 %) with a minor part via urine. Renal excretion in male animals of all test groups was half as much as that of the females, with correspondingly higher excretion in faeces. Large quantities of faecal radioactivity could be assigned to biliary excretion into the intestinal lumen, and enterohepatic re-circulation of the radioactivity was indicated.

Table 6.1-7. Cumulative excretion of radioactivity after oral administration of [phenyl-UL-^{14}C]tebuconazole expressed as % dose administered

Test No. **Dose** **Experiment** **Duration, sex**	**Test 1** **20 mg/kg** **expired air test** **72 h, male**	**Test 2** **2 mg/kg.** **bile-duct cannulation** **48 h, male***	**Test 3** **2 mg/kg** **single low dose** **72 h, male**	**Test 4** **2 mg/kg** **single low dose** **72 h, female**
Expired air (h)				
8	0.01	---	----	----
24	0.02	---	----	----
32	0.02	---	----	----
48	0.03	---	----	----
56	0.03	---	----	----
72	0.03	---	----	----
Urine (h)				
1	---	<0.01[4]	----	----
2	---	<0.01	----	----
3	---	0.97	----	----
4	1.02	2.36[4]	3.21	8.64
6	---	4.34	----	----
8	3.19	5.53	6.59	14.22[3]
12	---	6.68	----	----
18	---	7.10	----	----
24	13.36	7.26	13.95	28.12
30	---	7.33	----	----
32	14.45	---	14.93	29.91
36	---	7.37	----	----
42	---	7.39	----	----
48	15.77	7.40	15.91	32.02
56	15.96	---	16.07	32.45
72	16.18	---	16.23	32.89
Bile (h)				
1	----	15.03	----	----
2	----	39.24	----	----
3	----	52.83	----	----
4	----	61.66	----	----
6	----	72.70[4]	----	----
8	----	85.82	----	----
12	----	88.01	----	----
18	----	88.70	----	----
24	----	89.64	----	----
30	----	90.11	----	----
36	----	90.54	----	----
42	----	90.63	----	----
48	----	90.69	----	----
Faeces (h)				
24	62.66[3]	1.45[4]	71.13	52.19
48	74.74	1.50	79.91	60.93
56	74.86	----	81.28	61.00
72	75.81	----	82.11	62.48
Total sum of excretion	92.01	99.58	98.33	95.37

* One of the rats died about 31 h after administration, so that the results are based on only 4 animals.

[3] values based on 4 animals

[4] values based on 3 animals

Table 6.1-8. Cumulative excretion of radioactivity at time intervals expressed as % dose administered

Test No. **Dose** **Experiment**	**Test 5** **2 mg/kg [1]** **multiple low dose[1]**	**Test 6** **2 mg/kg [1]** **multiple low dose[1]**	**Test 9[2]** **20 mg/kg** **single high dose**	**Test 8** **20 mg/kg** **single high dose**

Duration, sex	72 h, male	72 h, female	72 h, male	72 h, female
Urine (h)				
4	1.85	5.10	1.86	2.10[3]
8	5.02	9.87	4.99	7.95
24	12.78	25.80	14.39	23.29
32	13.94	28.53	15.33	25.21
48	14.50[3]	31.13	16.56	27.82
56	14.75[3]	31.69	16.72	28.31
72	15.00[3]	32.33	16.97	28.80
Faeces (h)				
24	64.33[3]	47.07	63.81	50.82
48	75.00	58.70	77.30	60.84
56	76.89	61.16	77.68	61.30
72	78.77	61.46	78.73	62.73
Total sum of excretion	95.35	93.79	95.70	91.53

[1] one radiolabelled dose after 14 daily non-labelled doses at 2 mg/kg bw
[2] repetition of test 7
[3] : values based on 4 animals
[4]: values based on 3 animals
* in test 2, one of the animals died about 31 h after administration.

Conclusions
An almost complete absorption of tebuconazole was observed in this study. Tebuconazole was primarily eliminated by the bile and in a minor part via the urine. Oral absorption was > 98 % based on urinary (7.4 %) and biliary (90.9 %) excretion within 48 hours.

The test compound was rapidly absorbed from the GIT of rats in all test groups indicated by short t_{max} values of the plasma levels between 0.33 and 1.7 hours. The terminal half-life of plasma residues (t1/2 = 31.9 and 52.5 h) and mean residence time of the radioactivity in the plasma (MRT 26.9 to 48.6 h), were short in relation to the observation period (72 h). Male animals showed a larger AUC than female animals (by a factor of 1.7 - 3), which led to correspondingly lower total CL (clearance) values in all male rats.

Overall, tebuconazole was rapidly and well distributed into organs and tissues with highest levels found in the liver. Tebuconazole did not accumulate in any organ or tissue after oral administration and was rapidly excreted. Radiolabelled residues in tissues and organs were low at termination (<1.5 %), but generally higher in male rats compared to females.

Excretion of the radioactivity was a fast and complete process with 91.5 and 98.4 % of the administered dose excreted with the urine and faeces within 72 hours in all groups. The major route of excretion was the faecal route. Only 0.032 % of the administered dose was expired as $^{14}CO_2$ and other ^{14}C-labelled volatile compounds.

B.6.1.1.2. *Distribution of radioactivity following oral exposure in the rat (single dose of 20 mg/kg bw)*

Previous evaluation:	In Tebuconazole DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.1.1.2/01
Study title	[Phenyl-UL-^{14}C] HWG 1608: Whole-body autoradiographic distribution of the radioactivity in the rat
Test substance	HWG 1608; phenyl-UL-^{14}C- labelled; specific activity: 84.4 µCi/mg
Purity (%) **Batch no.**	99.5 (specific activity: 84.4 µCi/mg) APF 13028500
Test animals	Male Wistar (BOR:WISW) rats
Groups	Total of 7 animals: six were treated with radiolabelled test substance and one was treated with non-radioactively

	labelled test substance to check for chemographic effects of the parent compound as compared to the X-ray film emulsion
Dose	20 mg/kg bw
Route	Oral/gavage
Vehicle	0.5% aqueous tragacanth gel solution
GLP	Yes
Guideline	OECD 417
Deviation	The following deviations from the OECD-Guideline 417 (2010) occurred: - quantities of test substance and metabolites collected in excreta not investigated/reported
Acceptable	Acceptable

Methods
The distribution of radiolabelled tebuconazole in rats was investigated by qualitative whole body autoradiography. [Phenyl-UL-^{14}C] labelled tebuconazole was administered in a 0.5% aqueous tragacanth gel solution at a dose level of 20 mg/kg bw to six male rats (plus one control animal). The animals were sacrificed 1, 4, 8, 24, 48 and 72 hours after administration of the test substance. After deep-freezing, sagittal sections of the animals (50 µm thick) were cut with a microtome and placed onto an X-ray film. The autoradiographs were visually inspected to estimate the relative concentrations of radio-activity in the various tissues and organs of the rats.

Results
After oral administration the radioactivity of the test substance was absorbed almost completely from the intestinal tract of the rat at a medium to high rate. One hour after administration radioactivity was detectable in all body tissues with the exception of the compact bone.

One hour after administration, radioactivity was detectable in almost all body tissues and organs with the exception of the compact bone. The radioactivity of the parent compound was unevenly distributed in the animal body. Very high concentrations were discernible in the contents of the gastrointestinal tract, in the preputial gland, as well as in some areas of the mucosa of the nose, the tongue and in the epithelium of the oesophagus. High concentrations were limited to the liver, the cortex of the adrenal gland, the infraorbital gland, and to the hair follicles of the dorsal skin. Mean concentrations were found in all fat tissues, in the brain and spinal marrow, in the lung, the pancreas, the salivary glands, the heart, the testes and in the kidneys. Low to very low concentrations were present in the papilla of the kidneys, the musculature, the bone marrow, the thymus, and in the skin. Very low concentrations were found in the blood indicating a high speed of distribution of the radioactivity in the animal body after resorption.

At the next two time points, 4 hours and 8 hours after administration, the relative distribution pattern of the radioactivity was only slightly altered by comparison. The partially very high concentrations in the area of periglottis and nasal mucosa discernible at the time 1 hour after administration had declined markedly. In the area of the gastrointestinal tract now very high concentrations were also detectable in the lumen of the large intestine by displacement of radioactivity from the small intestine. This finding points to the gradually starting elimination of the parent compound-related radioactivity with the faeces. Furthermore a slight increase of the concentrations was noticeable 8 hours after administration in the suprarenal cortex and in the blood in comparison to the other tissues and organs and on the other hand a decrease in the fatty tissues, the brain and the spinal marrow.

The above described relative distribution pattern of the radioactivity remained the same to a large extent also at the time points 24 and 48 hours after administration. Exceptions of this were the concentrations in the infraorbital gland, the hair follicles of the dorsal skin as well as the preputial gland which declined more than in the other tissues and organs. Also the concentrations in the fatty tissues, in the brain and the spinal marrow dropped further. On the other hand high concentrations were further present in the liver.

At the last time point, 72 hours after administration, no marked alterations of the relative distribution pattern of the radioactivity were discernible. At this time the radioactivity in the kidney was mainly concentrated in the area of the inner zone of the medulla. The blackening in the suprarenal cortex continued to be undiminished high and can be compared only with that in the lumen of the intestinal tract. Even at this late time the liver still showed an intensive grey tint. A medium concentration of radioactivity was still present in the blood. Elimination of parent compound-related radioactivity was not yet completed 72 hours after administration.

In addition, the evaluation of the autoradiographs alluded to a high biliary excretion combined with a long-lasting

enterohepatic circulation of radioactivity as well as to a relatively slow renal elimination rate with a low fraction excreted via the urine.

Conclusion
After oral administration the radiolabelled tebuconazole was absorbed from the intestinal tract of the rat at high rate: one hour after administration radioactivity was detectable in all body tissues except the compact bone. The very low level in the blood at this time indicates a very high distribution rate into the animal body after absorption. This result shows the good tissue permeability of the parent compound-related radioactivity.

The high to very high concentrations in the suprarenal cortex recognizable during the entire duration of the investigation are likely connected with the metabolism of the parent compound as this organ, besides the liver, is rich in enzymes which metabolize foreign substances.

The very high radioactivity level in the small intestine combined with a high level in the liver indicates a rapid elimination of tebuconazole-radioactivity in the bile. By comparison of the typical time period of gastrointestinal passage in the rat (30 - 40 hours), the very high levels in intestinal tract and liver detectable even 72 hours after administration, lead to the plausible assumption of a long-lasting, enterohepatic circulation of the radioactivity with repeated intestinal absorption and biliary excretion. The somewhat higher blood levels at later time points also indicated the re-absorbed radioactivity being redistributed again via the blood among the tissues and organs.

The extent of re-absorption and the duration of the enterohepatic circulation together with re-absorption processes in the kidney increase altogether the mean residence time of the radioactivity in the animal body and thus severely influence the rate of elimination from the animal body.

Based on the temporarily increased concentrations in the nasal mucous membranes and in the hair follicles of the dorsal skin it is to be presumed that for a short time the radioactivity is also eliminated to a small extent through the nasal mucus as well as via the accessory glands of the skin (hair follicles and sebaceous glands).

B.6.1.1.3. *Metabolism of tebuconazole following oral exposure in the rat (single doses of either 2 mg/kg bw or 20 mg/kg bw or a repeated low dose of 2 mg/kg bw/day for 15 days)*

Previous evaluation:	In Tebuconazole DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.1.1.3/01
Study title	FOLICUR: Metabolism part of the general metabolism study in the rat Addendum 1: FOLICUR: Metabolism part of the general metabolism study in the rat – Additional information requested by the EPA
Test substance	Tebuconazole as Folicur, HWG 1608 phenyl-UL-^{14}C- and triazol-3,5-^{14}C-labelled compounds
Purity (%) **Batch no.**	99.5 (phenyl-UL-^{14}C-HWG 1608: 84.4µCi/mg, triazol-3,5-^{14}C-HWG 1608: 56.5 µCi/mg) Not stated
Test animals	Male and female Wistar (BOR:WISW) rats
Groups	5/sex/dose
Dose	Single dose: 2 or 20 mg/kg bw; in some groups pre-treatment with 2 mg/kg bw of non-radioactive test substance
Route	Oral / gavage
Vehicle	0.5% aqueous tragacanth solution
GLP	Yes
Guideline	OECD 417
Deviation	The following deviations from the OECD-Guideline 417 (2010) occurred: none
Acceptable	Acceptable

Methods

The metabolism of the test substance after administration of either [phenyl-UL-^{14}C]-tebuconazole or [triazol-3,5-^{14}C]-tebuconazole to several groups of rats under varying experimental conditions was assayed. The dose groups were a single oral low dose of 2 mg/kg bw, a 14 daily single oral non-radioactive doses of 2 mg/kg, followed by a radioactive dose of 2 mg/kg on the 15th day and a single oral high dose of 20 mg/kg (Table 6.1-9.). In the main study [phenyl-UL-^{14}C]-tebuconazole was used. Each group consisted of 5 male and 5 female animals. In addition to these trials, the high dose of the triazole-labelled test substance was orally administered to both sexes. Purification and isolation of metabolites for identification and structure elucidation was done with samples of the excreta.

Table 6.1-9. Dose regimen and design of tests to investigate the metabolism of tebuconazole in rats

Group	Administered single dose of ^{14}C-tebuconazole, route	^{14}C-label	Number of rats and sex	Collection of samples during the test and at sacrifice	Duration
1	2 mg/kg bw, oral (single low dose)	phenyl	5 male	urine, faeces, skin, carcass and GIT*	72 hours
2	2 mg/kg bw, oral (single low dose)	phenyl	5 female	urine, faeces, skin, carcass and GIT	72 hours
3	2 mg/kg bw, oral after 14 daily non-labelled doses at 2 mg/kg bw (multiple low dose)	phenyl	5 male	urine, faeces, skin, carcass and GIT	72 hours
4	2 mg/kg bw, oral after 14 daily non-labelled doses at 2 mg/kg bw (multiple low dose)	phenyl	5 female	urine, faeces, skin, carcass and GIT	72 hours
5	20 mg/kg bw, oral (single high dose)	phenyl	5 male	urine, faeces, skin, carcass and GIT	72 hours
6	20 mg/kg bw, oral (single high dose)	phenyl	5 female	urine, faeces, skin, carcass and GIT	72 hours
1	20 mg/kg bw, oral (single high dose)	triazole	5 male	urine, faeces, skin, carcass and GIT	72 hours
2	20 mg/kg bw, oral (single high dose)	triazole	5 male	urine, faeces, skin, carcass and GIT	48 hours
3	20 mg/kg bw, oral (single high dose)	triazole	5 female	urine, faeces, skin, carcass and GIT	48 hours

*GIT = gastrointestinal tract

Results

Recovery

At least approx. 92.5 % (92.5 - 100.6 %) of the administered radioactivity was recovered in all tests 72 hours after oral administration. A summary of the radioactivity in percent of the administered dose found in excreta and body at sacrifice is presented in Tables 6.1-10. (phenyl label) and 6.1-11. (triazole label).

Table 6.1-10. Recovery of radioactivity in excreta and the body of rats following oral dosing of [phenyl-UL-^{14}C]tebuconazole, data presented as % of dose administered

Test no. Dose, route Experiment Duration, sex	Trial 1 2 mg/kg, oral single low dose 72 hours, male	Trial 2 2 mg/kg oral single low dose 72 hours, female	Trial 3 2 mg/kg, oral. multiple low dose[1] 72 hours, male	Trial 4 2 mg/kg, oral multiple low dose[1] 72 hours, female	Trial 5 20 mg/kg, oral. single high dose 72 hours, male	Trial 6 20 mg/kg, oral single high dose 72 hours, female
Urine	14.6	33.6	16.8	31.4	14.5	24.1
Faeces	77.1	60.6	80.3	65.0	77.2	67.5
Sum excreta	91.7	94.2	97.1	96.4	91.7	91.6
Skin	0.0659	0.0415	0.1303	0.1300	0.1259	0.0473
Body w/o GIT	0.3063	0.2661	0.4797	0.5833	0.4850	0.4090

GIT	0.4419	0.3011	0.5781	0.4110	0.8833	1.3173
Total body	0.8	0.6	1.2	1.1	1.5	1.8
Balance	92.5	94.8	98.3	97.5	93.2	93.4

[1] one radiolabelled dose after 14 daily non-labelled doses at 2 mg/kg bw

Table 6.1-11. Recovery of radioactivity in excreta and the body of rats following oral dosing of [triazole-3,5-^{14}C]tebuconazole, data presented as % of dose administered

Test no. **Dose, route** **Experiment** **Duration, sex**	**Trial 1** **20 mg/kg, oral** **single high dose** **72 hours, male**	**Trial 2** **20 mg/kg, oral** **single high dose** **48 hours, male**	**Trial 3** **20 mg/kg, oral** **single high dose** **48 hours, female**
Urine	19.3	24.0	24.5
Faeces	77.2	70.7	72.7
Sum excreta	96.5	94.7	97.2
Total body	0.4	5.9	3.0
Balance	96.9	100.6	100.2

Rate of Excretion

The excretion of radioactivity after oral administration of [phenyl-UL-^{14}C] and [triazole-3,5-^{14}C]tebuconazole to nine groups of rats under varying experimental conditions was assayed.

In terms of retrieved radioactivity, more than 90 % of the renally, or faecally eliminated radioactivity, was excreted within 72 h after phenyl labelled tebuconazole (Table 6.1-12.) and within 48 h after triazole labelled tebuconazole (Table 6.1-12).

After oral administration of phenyl labelled tebuconazole, at sacrifice (72 hours after dosing), 60.6 - 80.3 % of the administered dose had been excreted with the faeces and 14.5 - 33.6 % with the urine. After administration of triazole labelled tebuconazole, at sacrifice (72 hours after dosing for trial 1 and 48 hours for trials 2 and 3) 70.7 - 77.2 % of the administered dose had been excreted with the faeces and 19.3 – 24.5 % with the urine.

There was no dose-dependence detectable with the phenyl-labelled compound, but a significant dependence on the animals' sex: Male animals excreted 15.5 to 17% with the urine, female animals 26 to 35%. Complementarily, the males showed a higher portion of excreted radioactivity in the faeces (77 to 80%) as compared to females (60 to 67%). These patterns were nearly identical to those in the biokinetic study (B.6.1.1.1/01).

In the trials using the triazole-labelled compound no sex difference in the excretion pattern was observed, with the female animals' pattern resembling that of the respective study with the phenyl-labelled compound.

Overall, excretion of the radioactivity was a fast and complete process with both the phenyl and triazole label. With both radioactive labels the major route of excretion was the faecal route (60.6 – 80.3 %) with a minor part via urine (14.5 - 33.6 %). Renal excretion in male animals of the phenyl test groups was half as much as that of the females, with correspondingly higher excretion in faeces. No sex differences in excretion pattern were observed with the triazole label.

Table 6.1-12. Cumulative excretion of radioactivity at time intervals expressed as % dose administered (phenyl-^{14}C label)

Test no. **Dose, route** **Experiment** **Duration, sex**	**Trial 1** **2 mg/kg, oral** **single low dose** **72 hours, male**	**Trial 2** **2 mg/kg oral** **single low dose** **72 hours, female**	**Trial 3** **2 mg/kg, oral.** **multiple low dose[1]** **72 hours, male**	**Trial 4** **2 mg/kg, oral** **multiple low dose[1]** **72 hours, female**	**Trial 5** **20 mg/kg, oral.** **single high dose** **72 hours, male**	**Trial 6** **20 mg/kg, oral** **single high dose** **72 hours, female**
Urine [hours]						
8	5.0	13.9	5.1	12.1	3.7	6.3

24	11.8	28.1	13.4	26.5	10.9	17.7
48	14.2	32.6	16.1	30.7	13.9	22.8
72	14.6	33.6	16.8	31.4	14.5	24.1
Faeces [hours]						
24	61.4	49.2	63.4	50.5	60.9	41.2
48	75.4	59.4	78.1	63.5	75.2	62.8
72	77.1	60.6	80.3	65.0	77.2	67.5
Sum excreta	91.7	94.2	97.1	96.4	91.7	91.6

[1] one radiolabelled dose after 14 daily non-labelled doses at 2 mg/kg bw

Table 6.1-13. Cumulative excretion of radioactivity at time intervals expressed as % dose administered (triazole-3,5-^{14}C label)

Test no. **Dose, route** **Experiment** **Duration, sex**	**Trial 1** **2 mg/kg, oral** **single high dose** **72 hours, male**	**Trial 2** **2 mg/kg, oral** **single high dose** **48 hours, female**	**Trial 3** **2 mg/kg, oral** **single high dose** **48 hours, male**
Urine [hours]			
8	4.5	6.3	8.8
24	14.6	19.2	20.1
48	18.7	24.0	24.5
72	19.3	-	-
Faeces [hours]			
24	62.0	53.0	61.5
48	75.6	70.7	72.7
72	77.2	-	-
Sum excreta	96.5	94.7	97.2

Metabolism

[Phenyl-UL-^{14}C]- and [triazole-3,5-^{14}C]tebuconazole were intensively metabolised in the rat. Eleven compounds, including the parent compound, were identified in urine and faeces (Tables 6.1-14. and 6.1-15.).

Regarding the excreta as a whole, tebuconazole-1-hydroxy (M03) and tebuconazole-carboxylic acid (M06) were the main metabolites in all test groups and amounted from 15.7 to 28.2% (M03) and from 14.1 to 36.2% (M06) of the administered radioactivity, with a slight tendency towards higher amounts in females. Sex-related differences between the test groups were found in the quantitative distribution of the some of the minor metabolites in the excreta. One compound, tebuconazole-1,5-di-OH-glucuronide (M12) was detected in the excreta of male animals only and amounted from 0.7 to 1.3% of the administered radioactivity. Two further compounds, tebuconazole-1,5-dihydroxy (M04) and tebuconazole-ketocarboxylic acid (M07) were detected in significant higher amounts in the excreta of the males than in those of the females. The corresponding values for the males were 1.3 to 5.6% (M04) and 2.3 to 5.6% (M07) compared to 0.4 to 0.8% (M04) and 0.8 to 1.1% (M07) of the administered radioactivity in the females. Two compounds were found in greater amounts in the excreta of the females. Tebuconazole-1-hydroxysulfate (M10) amounted from 2.0 to 2.3%, tebuconazole-1-OH-glucuronide (M11) from 3.0 to 4.8% of the administered radioactivity in females. Both compounds were detected in the excreta of the males in amounts of less than one tenth of these values. Two further compounds, tebuconazole-o-hydroxy (M02) and tebuconazole-desmethyl (M14) were detected in minor amounts and showed no significant dose- or sex-dependent differences.

Neither the dose level nor the pre-treatment showed a significant influence on the metabolic pattern in any of the dose groups.

Five unidentified compounds were detected in all dose groups, none of them exceeding 3.5% of the administered radioactivity. Sex-related differences between the test groups were detected in the distribution of these compounds. In general, females excreted less than half as much of these compounds than males.

In total, 20.3 % to 41.6 % of the administered dose remained unidentified after extraction (of faeces) in each

matrix. This unidentified activity included the above mentioned unidentified compounds, background activity not assigned to specific metabolites or fractions and post extraction solids in the case of faeces (Tables 6.1-14. and 6.1-15.).

The rate of identification was high, after application of 2.0 or 20.0 mg [phenyl-UL-^{14}C]-labelled tebuconazole/kg bw, ranging between 51.0 % and 71.3 % of the administered radioactivity. The identification balance did not take into account the amount of 1,2,4-triazole ("free triazole") found in the study with [triazole-3,5-14C]-labelled tebuconazole. For the total material balance, the amount of identified radioactivity should include the figures for 1,2,4-triazole as well. The proposed metabolic pathway is shown in Figure 6.1-3.

Comparison of the metabolic profiles of faeces extracts of both labels showed the same patter, therefore triazole-labelled metabolites from faeces extracts were not quantified in this study. Comparison of the metabolic profiles in urines of the animals treated with differently labelled tebuconazole raised one significant difference. One additional metabolite, identified as 1,2,4-triazole (M26), amounted to 5.4% in males and 1.6 % of the administered radioactivity in females. The metabolic profiles revealed similar sex-related differences as already observed in the animals treated with the phenyl-labelled test substance (Tables 6.1-14. and 6.1-15.).

Overall, eleven compounds, including the parent compound, were identified in urine and faeces. Tebuconazole was intensively metabolized: the unchanged parent compound was found at a maximum portion of 0.5 – 2.2 % of dose. M03 and M06 were major metabolites in all test groups with a slight tendency towards higher amounts in females. No significant differences in the metabolic pattern of the faeces extracts of both labels were observed. Following administration of triazole labelled tebuconazole, an additional metabolite, M26 (1,2,4-triazole) was identified, amounting to 5.4/1.6 % of the administered dose in the urine of male/female rats. Neither the dose level nor the pre-treatment showed a significant influence on the metabolic pattern in any of the dose groups.

Table 6.1-14. Quantitative distribution of metabolites in urine and faeces in % of the administered dose 72 hours (or 48 hours) after oral administration of [phenyl-UL-^{14}C]- or [triazole 3,5-^{14}C]-labelled tebuconazole

Test No.	**1**			**2**			**3**			**4**		
Dose	**2 mg/kg**			**2 mg/kg**			**2 mg/kg**			**2 mg/kg**		
Experiment	**single low dose**			**single low dose**			**multiple low dose[1]**			**multiple low dose[1]**		
^{14}C-radiolabel	**phenyl-UL**			**phenyl-UL**			**phenyl-UL**			**phenyl-UL**		
Time interval, Sex	**72h, male**			**72h, female**			**72h, male[1]**			**72h, female[1]**		
Excreta	**urine**	**faeces**	**total**	**urine**	**faeces**	**total**	**urine**	**faeces**	**total**	**urine**	**faeces**	**total**
Tebuconazole	-	0.5	0.5	-	0.6	0.6	-	0.7	0.7	-	0.5	0.5
M02	-	2.4	2.4	-	3.1	3.1	-	3.4	3.4	-	3.1	3.1
M03	0.1	15.6	15.7	0.3	18.6	18.9	0.1	16.7	16.8	1.8	19.9	21.7
M04	-	1.3	1.3	-	0.5	0.5	-	2.2	2.2	-	0.8	0.8
M06	1.8	30.8	32.6	12.5	23.7	36.2	1.1	26.0	27.1	11.5	23.8	35.3
M07	1.5	1.9	3.4	1.0	0.1	1.1	2.4	3.1	5.6	0.8	-	0.8
M10	-	-	-	2.0	-	2.0	0.1	-	0.1	2.1	-	2.1
M11	0.5	-	0.5	4.8	-	4.8	0.3	-	0.3	3.0	-	3.0
M12	1.3	-	1.3	-	-	-	0.7	-	0.7	-	-	-
M14	-	0.6	0.6	-	0.7	0.7	-	0.7	0.7	-	0.9	0.9
M26	-	-	-	-	-	-	-	-	-	-	-	-
Total identified[2]	5.2	53.1	58.4	20.6	47.3	67.9	4.7	52.8	57.5	19.2	49.0	68.2
Sum unknowns (largest unknown)	1.4 (1.4)	5.3 (2.0)	6.8 (2.0)	1.1 (1.1)	1.7 (0.7)	2.7 (1.1)	1.2 (1.2)	4.7 (1.5)	5.9 (1.5)	1.0 (1.0)	1.7 (0.6)	2.6 (1.0)
Not assigned[3]	8.1	11.7	19.8	11.8	8.5	19.9	10.9	15.1	26.0	11.2	10.1	21.4
solids	-	6.9	6.9	-	3.8	3.7	-	7.7	7.7	-	4.3	4.3
Total radioactivity excreted	14.7	77.0	91.7	33.5	61.3	94.8	16.8	80.3	97.1	31.4	65.1	96.5

[1] : pre-treated with 2.0 mg/kg bw daily for 14 days

Test No.	**1**			**2**			**3**			**4**		
Dose	**2 mg/kg**			**2 mg/kg**			**2 mg/kg**			**2 mg/kg**		
Experiment	**single low dose**			**single low dose**			**multiple low dose[1]**			**multiple low dose[1]**		
^{14}C-radiolabel	**phenyl-UL**			**phenyl-UL**			**phenyl-UL**			**phenyl-UL**		
Time interval, Sex	**72h, male**			**72h, female**			**72h, male[1]**			**72h, female[1]**		
Excreta	**urine**	**faeces**	**total**	**urine**	**faeces**	**total**	**urine**	**faeces**	**total**	**urine**	**faeces**	**total**

[2] : any lack of correspondence between the sum of the individual values and the "total" values is due to rounding
[3] : radioactivity not in discrete fractions
For chemical names and codes see Table 6.1-16 or B.6.1.3., Figure 6.1-3.

Table 6.1-15. Quantitative distribution of metabolites in urine and faeces in % of the administered dose 72 hours (or 48 hours) after oral administration of [phenyl-UL-^{14}C]- or [triazole 3,5-^{14}C]-labelled tebuconazole

Test No.	**5**			**6**			**2**			**3**		
Dose	**20 mg/kg**			**20 mg/kg**			**20 mg/kg**			**20 mg/kg**		
Experiment	**single high dose**			**single high dose**			**single high dose**			**single high dose**		
^{14}C-radiolabel	**phenyl-UL**			**phenyl-UL**			**triazole-3,5**			**triazole-3,5**		
Time interval, Sex	**72 h, male**			**72 h, female**			**48 h, male**			**48 h, female**		
Excreta	**urine**	**faeces**	**total**	**urine**	**faeces**	**total**	**urine**	**faeces[4]**	**total**	**urine**	**faeces[4]**	**total**
Tebuconazole	-	2.2	2.2	-	0.5	0.5	-	-	-	-	-	-
M02	-	4.7	4.7	-	5.1	5.1	-	-	-	-	-	-
M03	-	19.7	19.7	0.2	28.0	28.2	2.2	-	-	0.3	-	-
M04	-	5.6	5.6	-	0.4	0.4	-	-	-	-	-	-
M06	0.7	13.4	14.1	8.2	21.6	29.8	1.6	-	-	9.7	-	-
M07	2.3	-	2.3	1.0	-	1.0	3.4	-	-	0.7	-	-
M10	0.1	-	0.1	2.3	-	2.3	0.2	-	-	2.7	-	-
M11	0.2	-	0.2	3.7	-	3.7	0.3	-	-	2.9	-	-
M12	1.0	-	1.0	-	-	-	0.5	-	-	0.2	-	-
M14	-	1.1	1.1	-	0.3	0.3	-	-	-	-	-	-
M26	-	-	-	-	-	-	5.4	-	-	1.6	-	-
Total identified[2]	4.3	46.7	51.0	15.4	55.9	71.3	13.6		-	18.1	-	-
Sum unknowns (largest unknown)	1.0 (1.0)	9.7 (3.5)	10.7 (3.5)	0.7 (0.7)	1.5 (0.8)	2.2 (0.8)	-	-	-	-	-	-
Not assigned[3]	9.1	11.3	20.4	7.9	5.8	13.7	10.2	-	-	6.4	-	-
solids	-	9.5	9.5	-	4.4	4.4	-	-	-	-	-	-
Total radioactivity excreted	14.4	77.2	91.6	24.0	67.6	91.6	23.8	70.3	94.1	24.5	72.6	97.0

[1] : pre-treated with 2.0 mg/kg bw daily for 14 days
[2] : any lack of correspondence between the sum of the individual values and the "total" values is due to rounding
[3] : radioactivity not in discrete fractions
[4] : no values reported for faeces since metabolic pattern of rats treated with phenyl- and triazole label almost identical
For chemical names and codes see Table 6.1-16 or B.6.1.3., Figure 6.1-3.

Conclusions

Overall, excretion of the radioactivity was a fast and complete process with both the phenyl and triazole label. With both radioactive labels the major route of excretion was the faecal route (60.6 – 80.3 %) with a minor part via urine (14.5 - 33.6 %). Renal excretion in male animals of the phenyl test groups was half as much as that of the females, with correspondingly higher excretion in faeces. No sex differences in excretion pattern were observed with the triazole label. Less than 6 % of the applied radioactivity could be detected in the total body at sacrifice.

Eleven compounds, including the parent compound, were identified in urine and faeces. Tebuconazole was intensively metabolized: the unchanged parent compound was found at a maximum portion of 0.5 – 2.2 % of dose. M03 (tebuconazole-1-hydroxy up to 28.2 %) and M06 (tebuconazole-carboxylic acid up to 36.2 %) were major metabolites in all test groups with a slight tendency towards higher amounts in females. Minor metabolites were identified in urine and faeces as M02 (tebuconazole-o-hydroxy), M04 (tebuconazole-1,5-dihydroxy), M07 (tebuconazole-ketocarboxylic acid), M10 (tebuconazole-1-hydroxysulfate), M12 (tebuconazole-1,5-di-OH-glucuronide), and M14 (tebuconazole-desmethyl) showing some sex-dependent excretion behaviour. Following administration of triazole labelled tebuconazole, an additional metabolite, M26 (1,2,4-triazole) was identified, amounting to 5.4/1.6 % of the administered dose in the urine of male/female rats. Neither the dose level nor the pre-treatment showed a significant influence on the metabolic pattern in any of the dose groups.

Distinct sex differences were seen in the metabolic pattern of tebuconazole which mainly involves phase-1 reactions, i.e. oxidations, resulting in hydroxy, carboxy, triol and ketoacid metabolites followed by conjugation with glucuronic and sulfuric acid (phase 2 reactions). Female rats showed preferably simple oxidation products like M03 (tebuconazole-1-hydroxy) and M06 (tebuconazole-carboxylic acid), followed by conjugation and only a minor cleavage of the triazole moiety. Male animals exhibit a slightly more complex metabolic behaviour by further oxidising the primary metabolites to M04 (tebuconazole-1,5-dihydroxy) and M07 (tebuconazole-ketocarboxylic acid), and additionally by a more pronounced cleavage of the triazole moiety.

In the following table, different designations of the tebuconazole metabolites are compiled.

Table 6.1-16. Different designations of the tebuconazole metabolites

No in LoM (Doc N3)	Name in the original report	Common name	Main ~~Major~~ metabolite (total excretion urine + faeces)
a.s.	HWG 1608 “parent”	Tebuconazole, HWG 1608	0.5 –2.2 % Parent
M02	ECW 4882 “phenol”	tebuconazole-o-hydroxy	2.6 – 5.1 % Minor
M03	EWC 4884, HWG 2061 “diol”	tebuconazole-1-hydroxy	15.7 - 28.2 % ~~Major~~ Minor (0.1-2.2% in urine)
M04	ECW 4886 “triol”	tebuconazole-1,5-dihydroxy	0.5 – 5.6 % Minor
M06	ECW 4885, HWG 2443 “acid”	tebuconazole-carboxylic acid	14.1 - 36.2 % Major in female (11 % in urine)
M07	EWC 4881, ECW 4873 “keto acid”	tebuconazole-5-keto-hydroxy acid	0.8 – 5.6 % Minor
M10	ECW 4390 “diol sulfate”	tebuconazole-1-hydroxysulfate	0.1 – 2.3 % Minor
M11	ECW 4393 2/2 “diol glucuronide”	tebuconazole-1-OH-glucuronide	0.2 – 4.8 %
M12	ECW 4908 “triol-glucuronide””	tebuconazole-1,5-di-OH-glucuronide	0.7 – 1.3 % Minor
M14	HWG 2251 (assumed to be formed by microbial decarboxylation of M06 in the intestine under reductive conditions)	tebuconazole-desmethyl	0.3 – 1.1 % Minor
M26	ECW 4895/2 “triazole”	1,2,~~45~~-triazole	1.6 – 5.4% Minor

B.6.1.1.4. *Publications of relevance to toxicokinetics*

Previous evaluation:	None – publication submitted for the purpose of renewal

Study ID	B.6.1.1.4/01
Author(s)	Mercadante *et al*., 2014
Study title and Journal	Identification and quantification of metabolites of the fungicide tebuconazole in human urine. Chemical Research in Toxicology, 2014, 27, 1943-1949
Test substance	Workers exposed to tebuconazole
Purity (%) Batch no.	Not applicable
GLP	No
Guideline	Not applicable
Deviation	Not applicable
Reliability	Reliable with restrictions
Relevance to hazard assessment	Relevant

Methods and findings
In this study a method was developed to identify and quantify metabolites of tebuconazole in human urine. Samples from seven vineyard workers exposed to tebuconazole (TEB) were submitted to liquid chromatography interfaced with a triple quadrupole mass spectrometer, equipped with an electron spray source, and a linear ion trap to gain a profile of candidate metabolites. Based on the presence of the ion m/z 70 in the MS/MS spectra, which corresponds to protonated triazole (a specific moiety of TEB), and the isotopic pattern of the molecular ions, typical of molecules with one chlorine atom, hydroxyl and carboxyl derivatives of TEB, that is, TEB-OH and TEB-COOH, were identified as major metabolites, both as free molecules and as glucuronide (Glc) conjugates. The mean molar fractions were 0.67, 0.13, 0.13, and 0.07 for TEB-O-Glc, TEB-OH, TEB-COO-Glc, and TEB-COOH. Urine samples were submitted to hydrolysis with β-glucuronidase, and the free compounds were quantified in the presence of deuterated TEB (TEBd6) as the internal standard (IS), by multiple reaction monitoring (MRM) mode. The assay was linear in the ranges of 0.2−600 μg/L and 0.1−240 μg/L for TEB-OH and TEB-COOH, respectively; precision, accuracy, and the limit of quantification (LOQ) were <3.1%, 98−103%, and 0.3 μg/L for both analytes. An evaluation of matrix effects showed that the use of TEB-d6 controlled these sources of bias. The urinary levels of TEB-OH and TEB-COOH in specimens collected from farmers exposed to TEB ranged from 10 to 473 and from 3 to 159 μg/L, respectively.

Conclusion
Hybrid triple quadrupole/linear ion trap mass spectrometry is suitable to investigate the metabolism of environmental contaminants, such as the pesticide TEB, in the human body by direct analysis of urine samples of exposed subjects, without requiring an ad hoc experiment with volunteers. In this work TEB-OH and TEB-COOH were identified, both as free molecules and as glucuronide conjugates, as the main metabolites of TEB. TFLC coupled with LC−MS/MS allowed to set an analytical assay with minimal sample preparation and to achieve good analytical performances for routine analysis of specimens of agricultural workers.

(Merchadante *et al*., 2014)

Previous evaluation:	None – publication submitted for the purpose of renewal

Study ID	B.6.1.1.4/02
Author(s)	Zhu *et al*., 2007
Study title and Journal	Stereoselective degradation kinetics of Tebuconazole in rabbits. Chirality, 19(2), 141-147.
Test substance	Tebuconazole racemic
Purity (%) Batch no.	>99%

GLP	No
Guideline	Not applicable
Deviation	Not applicable
Reliability	Reliable with restrictions – measurements were done at different time points from 1 animal only.
Relevance to hazard assessment	Relevant

Methods
Racemic tebuconazole was dissolved in ethanol and administered at 30 mg/kg bw by iv injection to male Japanese white rabbits (number not given). Blood samples were collected at 0 (blank), 5, 15, 30, 60, 120, 240, and 480 min after treatment, and one time point corresponds to one animal. The heart, kidney, liver, lung, fat, muscle, spleen, and brain of each rabbit were also collected. Blood and tissue samples were analysed for levels of the two enantiomers.

Findings and conclusions
Following single iv administration to rabbits, stereoselective disposition of the (-)-and (+)-enantiomers of racemic tebuconazole was observed. Concentration of the (+)-enantiomer in plasma decreased more rapidly than that of the (-)-enantiomer. Plasma protein binding and stereoselective plasma protein binding may contribute to these differences. However, chiral conversion of tebuconazole in plasma may also play a role. Stereoselective degradation of tebuconazole enantiomers in some tissues was also observed. This could be due to chiral inversion of the two enantiomers in plasma, but also stereoselective distribution of (+)- and (-)-tebuconazole in tissues. The applicant notes that the data supporting different degradation behaviour of the 2 isomers are not convincing, since the curves of both isomers in plasma and tissues did not show a very different behaviour of the 2 isomers, especially after longer times.

(Zhu *et al*., 2007)

Previous evaluation:	None – publication submitted for the purpose of renewal

Author(s)	Shen *et al.*, 2011
Study title and Journal	Stereoselective degradation of tebuconazole in rat liver microsomes. Chirality, 24, 67-71.
Test substance	Tebuconazole racemic
Purity (%) Batch no.	>99%
GLP	No
Guideline	Not applicable
Deviation	Not applicable
Reliability	Reliable with restrictions – measurements were done at different time points from 1 animal only.
Relevance to hazard assessment	Relevant with restrictions

Methods
The aim of this study was to assess the stereoselectivity of the two tebuconazole enantiomers in an *in vitro* system (rat liver microsomes). Racemic tebuconazole was dissolved in ethanol and added to rat liver microsomes at 15 µM for up to 90 min. Samples were analysed for levels of the two enantiomers and rate of metabolism.

Findings and conclusions
In this *in vitro* system, the degradation of the S-(+)tebuconazole was faster than that of the R-(-)tebuconazole, in line with the results seen *in vivo*.

(Shen *et al*., 2011)

B.6.1.2. *In vitro* metabolism studies

Two *in vitro* interspecies comparative metabolism studies were conducted with the parent substance tebuconazole radiolabelled in the triazole moiety using either mouse, rat and human liver S9 fractions, or mouse, dog, rat, and human hepatocytes. Additionally, plasma protein binding of [phenyl-UL-^{14}C] tebuconazole was investigated in plasma of mouse, rat, rabbit, dog and human. These *in vitro* studies were submitted for the purposes of renewal by the Bayer Task Force. No *in vitro* interspecies comparative metabolism studies were submitted by the EU Tebuconazole Task Force.

B.6.1.2.1. *Comparative* in-vitro *metabolism of [triazole-UL-^{14}C]tebuconazole in mouse, rat and human liver S9 fractions*

Previous evaluation:	None – submitted for the purpose of renewal (study owned by Bayer Task force)

Study ID	B.6.1.2/01
Study title	[triazole-UL-^{14}C] tebuconazole: Metabolic stability and profiling in liver S9 fractions from the mouse [triazole-UL-^{14}C] tebuconazole: Metabolic stability and profiling in liver S9 fractions from rat and human for inter-species-comparison
Test substance	Tebuconazole (radiolabelled: triazole-UL-^{14}C]tebuconazole)
Purity (%) **Batch no.**	> 98 (HPLC) KML 9879
Test system	S9 liver fractions from male and female mice (Strain CD1), Wistar rats and humans
Groups	1 or 10 µM test substance, with or without NADP cofactor containing glucose-6-phosphate dehydrogenase. Positive control 14C-testosterone
Dose	1 or 10 µM
Route	Incubated one and two hours at 37°C
Vehicle	phosphate buffer K_2HPO_4 (50mM + 1mM EDTA, pH 7.4)
GLP	Yes
Guideline	n/a
Deviation	n/a
Acceptable	Acceptable

Methods

Pooled liver S9 enzyme fractions of mice (CD1), rats (Wistar) or humans of both genders, with a protein concentration of 1.0 mg protein/mL, were incubated in a phosphate buffer (pH 7.4) with 1 or 10 µM [triazole-UL-14C]tebuconazole. The enzymatic activity of the incubation was started by addition of the NADPH regeneration system (NADP cofactor containing glucose-6-phosphate dehydrogenase) to the mixture and was stopped after 1 or 2 hours by addition of 100 µL acetonitrile. Control incubations with the same concentration of the test substance tebuconazole were conducted without NADPH to show the stability of tebuconazole in the buffer solution. The metabolic activity of the enzyme fractions was proven by the metabolisation of the positive control substance 14C-testosterone. All incubations were performed at 37 ± 1°C using a water bath for temperature control. The incubation vessels were gently shaken at a frequency of approx. 120 rpm.

Results

Radioactivity balance

The radioactivity recovered after 1 and 2 hours was compared to the applied radioactivity. The recoveries were very good in all conditions and amounted to 82.2 – 102.8 % of the applied radioactivity after 1 hour and to 78.6 – 109.6 % of the applied radioactivity after 2 hours for all incubates of [triazole-UL-^{14}C]tebuconazole at both concentrations. For the control substance ^{14}C-testosterone the recoveries accounted for 92.3 - 104.0 % of the applied radioactivity after 1 hour and for 92.2 - 105.7 % of the applied radioactivity after 2 hours.

Metabolic conversion of the test substance [triazole-UL-^{14}C]tebuconazole

The possible metabolic conversion of [triazole-UL-^{14}C]tebuconazole was investigated at 1 and 10 µmolar solutions in liver S9 fractions from mouse, rat and human of both genders (Table 6.1-17.). Additionally, control tests were conducted with incubations of [triazole-UL-^{14}C]tebuconazole in a system without regeneration system (generating NADPH).

Table 6.1-17. Metabolic conversion of 1 and 10 µM [triazole-UL-^{14}C]tebuconazole in S9 liver fractions

Species/Test system	Relative amount of [triazole-UL-^{14}C]tebuconazole in the radiochromatogram [%]			Rate of conversion*	
Incubation period	0 h	1 h	2 h	1 h	2 h
Rat - 1 µM					
male	100.0	6.5	2.7	93.5	97.3
female	100.0	42.6	33.8	57.4	66.2
Rat - 10 µM					
male	100.0	85.4	83.9	14.6	16.1
female	100.0	91.4	86.0	8.6	14.0
Human - 1 µM					
male	100.0	87.9	81.0	12.1	19.0
female	100.0	100.0	90.6	0.0	9.4
Human - 10 µM					
male	100.0	98.5	98.6	1.5	1.4
female	100.0	99.4	98.5	0.6	1.5
Mouse - 1 µM					
male	100.0	96.3	96.2	3.7	3.8
female	100.0	95.2	93.8	4.8	6.2
Mouse - 10 µM					
male	100.0	100.0	100.0	0.0	0.0
female	100.0	98.2	98.5	1.8	1.5

* Rate of conversion = (rel. amount after 1, 2 h) / rel. amount of control at 2 h

Rat
Incubation of 1 µM [triazole-UL-^{14}C]tebuconazole with rat liver S9 fractions showed the highest metabolic transformation rate accounting for 66.2 % and 97.3 % for female and male rat liver fractions, respectively. Incubation of 10 µM [triazole-UL-^{14}C]tebuconazole with rat liver S9 fractions showed a lower metabolic transformation rate accounting for 14.0 % and 16.1 % for female and male rat liver fractions, respectively (Table 6.1-17.).

Human
Incubation of 1 µM [triazole-UL-^{14}C]tebuconazole with female and male human liver S9 fractions accounted for 9.4 % and 19.0 %, respectively. The lowest metabolic transformation rate was observed in incubations of 10 µM [triazole-UL-^{14}C]tebuconazole with human liver fractions accounting for 1.5 % and 1.4 % of the metabolic transformation for female and male human liver fractions, respectively (Table 6.1-17.).

Mouse
In the mouse liver S9 fraction incubates, [triazole-UL-^{14}C]tebuconazole was only slightly metabolised. The highest metabolic transformation rate was observed in liver fractions after incubation at 1 µM accounting for 3.8 – 6.2 % of the metabolic transformation. Transformation rates of 10 µM [triazole-UL-^{14}C]tebuconazole in female liver fraction incubates accounted for 1.5 %, no metabolic transformation at 10 µM was observed in male liver S9 fraction incubates (Table 6.1-17.).

Overall, the metabolic transformation rate was higher in rat liver fractions than in human and mouse liver fractions. In general, the metabolic transformation rate was higher after incubation of 1 µM [triazole-UL-^{14}C]tebuconazole with liver S9 fractions (3.8 – 97.3 %) compared to the incubations of 10 µM [triazole-UL-^{14}C]tebuconazole with liver S9 fractions (1.5 - 16.1 %).

Table 6.1-18. Summary of metabolite profiles of 1 and 10 µM [triazole-UL-^{14}C]tebuconazole in S9 liver fractions

Concentration of test item [triazole-UL-^{14}C] tebuconazole	Metabolite	Relative amount of [triazole-UL-^{14}C]tebuconazole in the radiochromatogram [%] *			
		1 h male	1 h female	2 h male	2 h female

Rat - 1 µM	**tebuconazole**	**6.5**	**42.6**	**2.7**	**33.8**
	R1	32.2	21.5	40.9	28.4
	R2	3.8	---	5.5	1.8
	R3	8.4	3.2	7.7	3.0
	R4	1.4	---	5.0	---
	R5	4.6	3.7	5.8	3.4
	R6	2.5	6.7	3.2	5.9
	R7	31.6	18.6	21.2	23.8
	R8	9.1	3.8	8.1	**---**
	R9	---	---	---	---
Rat - 10 µM	**tebuconazole**	**85.4**	**91.4**	**83.9**	**86.0**
	R1	---	---	---	---
	R2	---	---	---	---
	R3	---	---	---	---
	R4	---	---	---	---
	R5	---	1.7	0.3	2.7
	R6	1.1	6.9	0.7	11.3
	R7	8.3	---	9.0	---
	R8	2.4	---	3.0	---
	R9	2.8	---	3.1	---
Human - 1 µM	**tebuconazole**	**87.9**	**100.0**	**81.0**	**90.6**
	H7	12.1	---	19.1	9.4
Human - 10 µM	**tebuconazole**	**98.5**	**99.4**	**98.6**	**98.5**
	H7	1.6	0.6	1.5	1.5
Mouse - 1 µM	**tebuconazole**	**96.3**	**95.2**	**96.2**	**93.8**
	M7	3.7	4.8	3.8	6.2
	M9	---	---	---	---
	M10	---	---	---	---
Mouse - 10 µM	**tebuconazole**	**100.0**	**98.2**	**100.0**	**98.5**
	M7	---	0.8	---	0.7
	M9	---	0.5	---	0.6
	M10	---	0.5	---	0.3

--- not detected, below LOQ
* concentration of tebuconazole at 0 hours was 100.0% for both genders

In the incubates of 1 µM [triazole-UL-^{14}C]tebuconazole with male and female rat liver fractions, up to eight metabolites were detected (Table 6.1-18.). Metabolites R1 and R7 showed the highest abundance up to 40.9 and 31.6 % of the radioactivity, respectively. The further metabolites accounted for each ≤ 9.1 % of the radioactivity.

In the incubates of 10 µM [triazole-UL-^{14}C]tebuconazole with male rat liver fractions, up to five metabolites were detected and metabolite R7 showed the highest abundance up to 9.0 % of the radioactivity. The further metabolites accounted for each ≤ 3.1 % of the radioactivity.

For female rat liver fraction incubates at 10 µM only two metabolites were detected and metabolite R6 showed the highest abundance (up to 11.3 % of the radioactivity). The further metabolite R5 accounted for ≤ 2.7 % of the radioactivity.

Besides tebuconazole, only one metabolite (H7) was detected in incubates with human liver S9 fractions at 1 and 10 µM [triazole-UL-^{14}C]tebuconazole (accounting up to 19.1 % of the radioactivity). This metabolite showed the same retention time as R7 in the rat and M7 in the mouse.

Beside tebuconazole one prominent metabolite M7 and two minor metabolites, M9 and M10, were detected after 1 or 2 hours incubation of 1 and 10 µM [triazole-UL-^{14}C] tebuconazole with mouse liver S9 fractions. Metabolite M7 was the only metabolite detected in the incubates of 1 µM [triazole-UL-^{14}C]tebuconazole and showed the highest abundance up to 6.2 % of the radioactivity for male and female S9 liver fractions.

In the incubates of 10 µM [triazole-UL-^{14}C]tebuconazole with male mouse S9 liver fractions no metabolites were detected. Incubates of female S9 liver fractions at 10 µM showed the main metabolite M7 and the two further minor metabolites M9 and M10. Each metabolite accounted for ≤ 0.8 % of the radioactivity.

Overall, beside tebuconazole, two prominent metabolites R1 and R7 and up to six further metabolites were detected after 1 or 2 hours incubation of 1 and 10 µM [triazole-UL-14C]tebuconazole with rat liver S9 fractions. Only one metabolite (H7) was detected in incubates with human liver S9 fractions at both concentrations, and one prominent metabolite M7 and two minor metabolites, M9 and M10 were detected in mouse fractions.

Metabolites R7, H7 and M7 were detected at retention time of approximately 44 minutes. Therefore, no human unique metabolites were found in all incubates.

Due to the low amount of radioactivity, all detected metabolites were only characterised based on their chromatographic behaviour.

Metabolic conversion of the positive control ^{14}C-testosterone
Liver S9 fractions were metabolically active as demonstrated by the metabolic conversion of ^{14}C testosterone. Decreasing amounts of testosterone with increasing formation of radioactive metabolites (up to 26) demonstrated sufficient metabolic capability of the liver S9 fraction batches used in this study. The highest metabolic activities were measured for rat S9 liver fractions and amounted to 99.3 % for female and to 100 % for male rat liver fractions. Male and female human liver S9 fractions had a maximum metabolic activity of 34.8 % and 16.6 %, respectively. Male and female mouse liver S9 fractions had a maximum metabolic activity of 26.2 % and 14.7 % respectively.

Conclusion
[Triazole-UL-^{14}C]tebuconazole was incubated with male and female mouse, rat and human liver S9 fractions for one and two hours at 37°C. These comparative *in-vitro* tests suggested that tebuconazole is highly metabolised with rat liver fractions. Lower metabolic transformation of tebuconazole was detected in human liver fractions incubates and only slight metabolic transformation of tebuconazole was detected in mouse liver fractions incubates. The biotransformation of radiolabelled [triazole-UL-^{14}C] tebuconazole was generally higher after incubation at a concentration of 1 µM compared to 10 µM which indicated a possible inhibition of metabolic capability at higher concentrations. The enzymatic activity of each of the S9 enzyme fraction was demonstrated by a significant metabolic conversion of the positive control substance ^{14}C-testosterone. One major metabolite was formed similarly in all test systems, representing the metabolite R7, H7 or M7 detected at retention time of approximately 44 minutes. This metabolite amounted up to 41 %, 19 % or 6 % of the applied radioactivity for rat, human and mouse incubates, respectively. Comparison of the metabolic profiles showed that no unique human metabolite had been formed. Due to the low amounts of metabolites formed in these studies, it was not possible to characterise the metabolites in more detail.

B.6.1.2.2. ***Comparative* in-vitro *metabolism of [triazole-UL-^{14}C]tebuconazole in mouse, dog, rat, and human hepatocytes***

Previous evaluation:	None – submitted for the purpose of renewal (study owned by Bayer Task force)

Study ID	B.6.1.2/02
Study title	*In-vitro* metabolism of [triazole-UL-^{14}C]tebuconazole in mouse, dog, rat, and human hepatocytes
Test substance	Tebuconazole (radiolabelled: triazole-UL-^{14}C]tebuconazole)
Purity (%) **Batch no.**	> 99 (HPLC) KML 9879
Test system	hepatocytes of mouse (MH, 2 strains: NMRI and CD1), dog (DH), rat (RH) and human (HH)
Groups	Each hepatocyte type at each dose at 0, 1, 2, or 4 hours
Dose	1, 5, 10, and 20 µM
Route	Incubated 0, 1, 2 and 4 hours at 37°C
Vehicle	Williams E medium buffered with Carbogen® (5% CO2; 95% O2) (pH 7.4)
GLP	No

Guideline	n/a
Deviation	n/a
Acceptable	Acceptable

Methods

The comparative *in vitro* metabolism of [triazole-UL-^{14}C]tebuconazole was tested at four concentrations (1, 5, 10, and 20 µM) in freshly prepared or cryopreserved hepatocytes of mouse (MH, 2 strains: NMRI and CD1), dog (DH), rat (RH) and human (HH). The incubation times were 0, 1, 2, and 4 hours at 37 °C. The individual tests were stopped with acetonitrile and then centrifuged. Aliquots from the respective supernatants were afterwards analysed by HPLC with radiochemical detection for determination of the metabolic profiles. Parent compound and metabolites were identified in selected samples afterwards by LC-MS/MS.

The hepatocytes were incubated with phenacetin, repaglinide, diclofenac, dextro-methorphan, and midazolam at a concentration of 1 µM each to assess their metabolic capability (positive controls). The metabolic conversion of these compounds was analysed and calculated as intrinsic clearance (Cl_{int}).

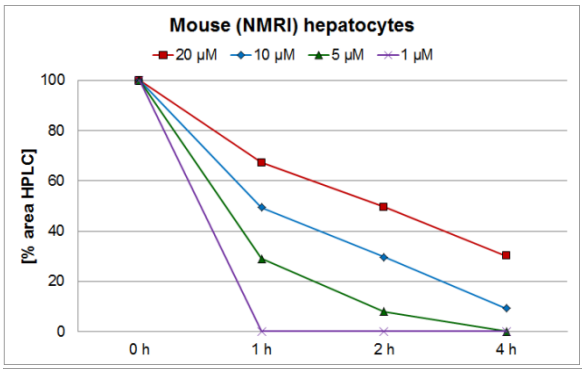
Results

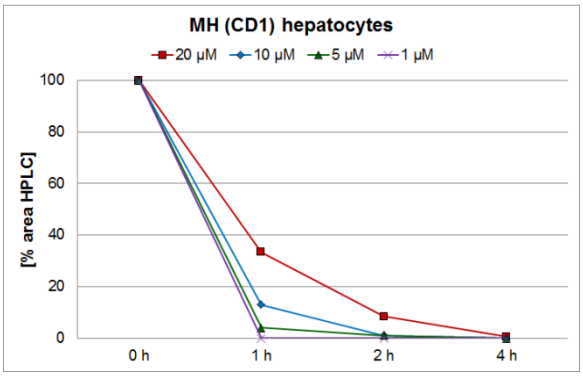
Viability testing of hepatocytes

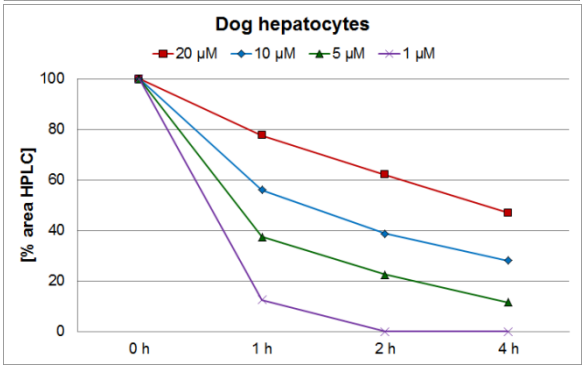
The hepatocytes were incubated with phenacetin, repaglinide, diclofenac, dextro-methorphan, and midazolam at a concentration of 1 µM each to assess their metabolic capability. All hepatocyte batches were metabolically competent and exhibited good activities.

Metabolite profiles of ^{14}C-tebuconazole

At a test concentration of 1 µM, the biotransformation rate was fast in all samples. At the latest after 2 hours the amount of unchanged parent compound dropped to values below the detection limit. Significantly higher amounts were measured with increasing test compound concentration presumably caused by inhibition of the metabolic capability (Figure 6.1-1).







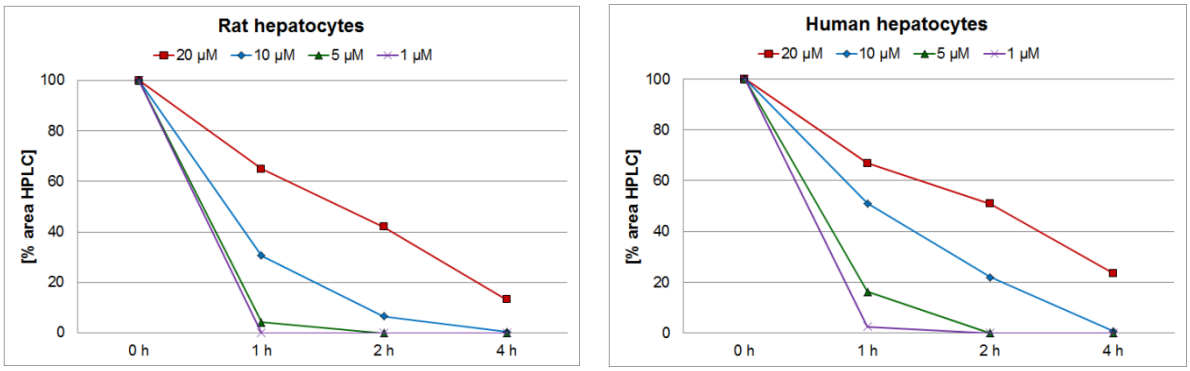


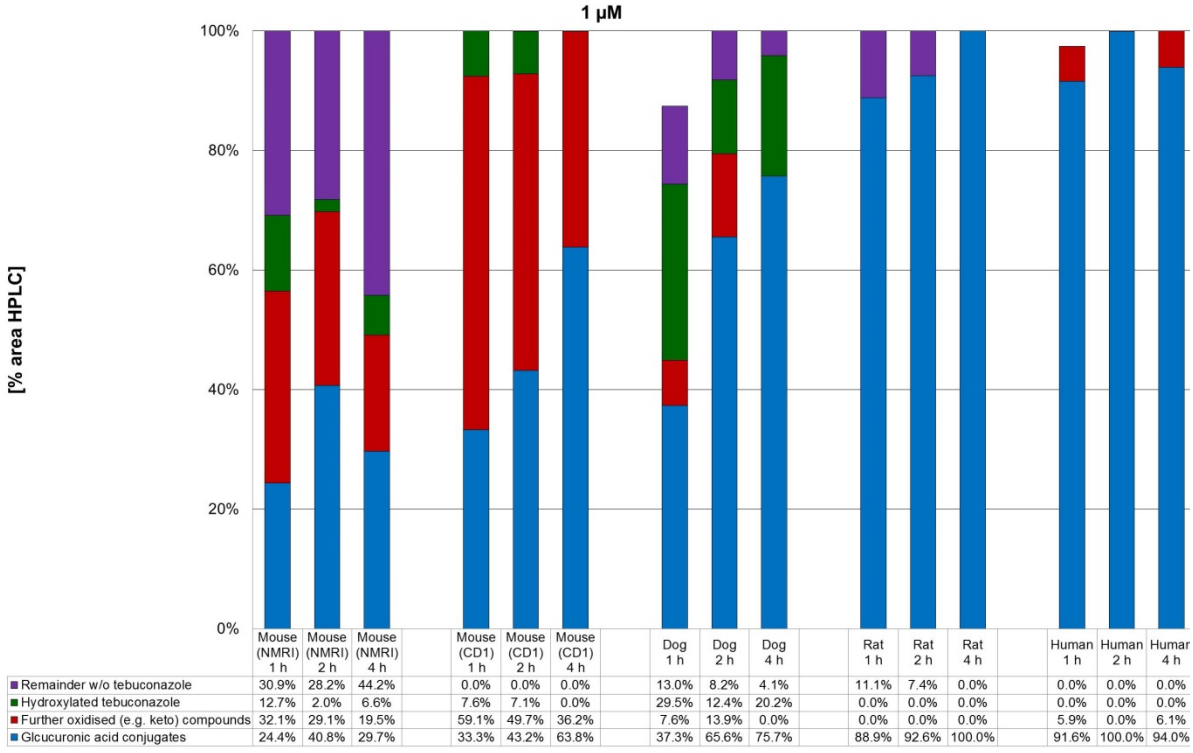
Figure 6.1-1. Metabolic profiles

With increasing test concentration an inhibition of metabolic capability was seen. This effect was seen in all the different hepatocyte species. The dog was the most sensitive, showing inhibition at 5µM whilst other species demonstrated minimal inhibition at this dose.

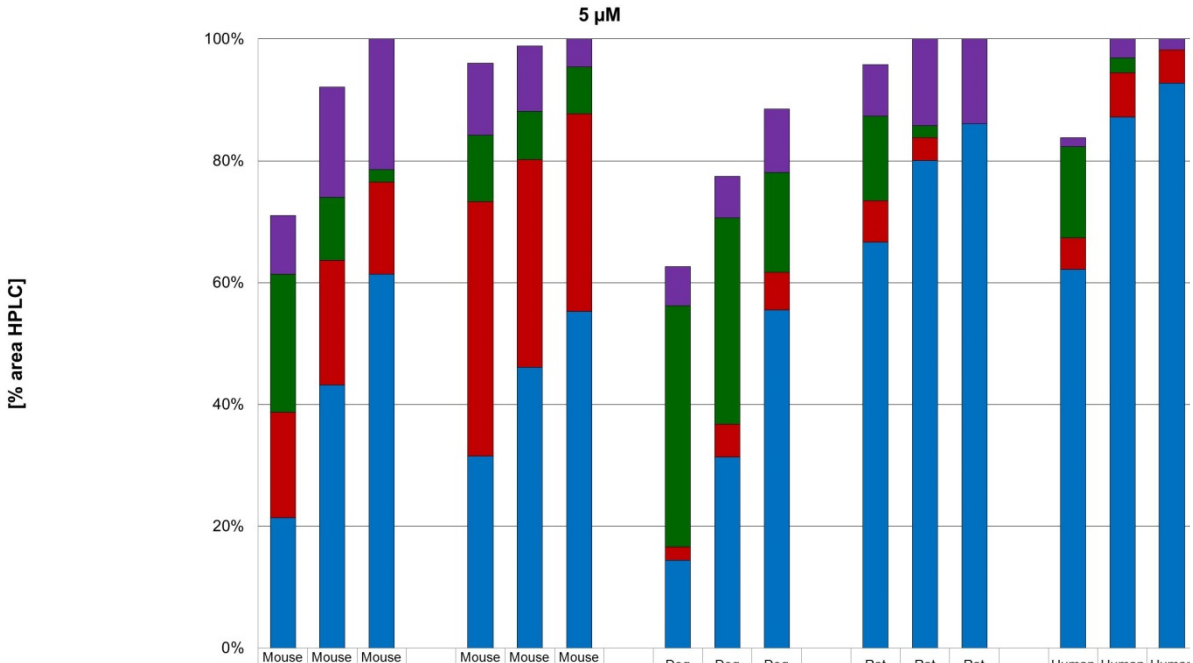
A large number of metabolites were observed in the tests. The principal metabolic reactions were presumably quite similar in all animal species. The main biochemical reactions were hydroxylation and oxidation at different sites of the molecule, and conjugation of hydroxylated metabolites mainly with glucuronic acid (abbreviated as GlcA). Other conjugation reactions with sulphate and glutathione also occurred. These, however, were of minor importance.

In order to obtain a simplified picture of the different metabolic reactions, the metabolites were classified into the following groups: remainder (unknown compounds) without the active ingredient (a.i.), further oxidation (i.e. keto compounds), hydroxylation, and conjugation with glucuronic acid. Missing percentages to 100 % (e.g. dog, 1 h) account for unchanged parent compound.

The diagrams for the 1 and 5 µM test concentrations are given below.



	Mouse (NMRI) 1 h	Mouse (NMRI) 2 h	Mouse (NMRI) 4 h	Mouse (CD1) 1 h	Mouse (CD1) 2 h	Mouse (CD1) 4 h	Dog 1 h	Dog 2 h	Dog 4 h	Rat 1 h	Rat 2 h	Rat 4 h	Human 1 h	Human 2 h	Humar 4 h
Remainder w/o tebuconazole	30.9%	28.2%	44.2%	0.0%	0.0%	0.0%	13.0%	8.2%	4.1%	11.1%	7.4%	0.0%	0.0%	0.0%	0.0%
Hydroxylated tebuconazole	12.7%	2.0%	6.6%	7.6%	7.1%	0.0%	29.5%	12.4%	20.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Further oxidised (e.g. keto) compounds	32.1%	29.1%	19.5%	59.1%	49.7%	36.2%	7.6%	13.9%	0.0%	0.0%	0.0%	0.0%	5.9%	0.0%	6.1%
Glcucuronic acid conjugates	24.4%	40.8%	29.7%	33.3%	43.2%	63.8%	37.3%	65.6%	75.7%	88.9%	92.6%	100.0%	91.6%	100.0%	94.0%



	Mouse (NMRI) 1 h	Mouse (NMRI) 2 h	Mouse (NMRI) 4 h	Mouse (CD1) 1 h	Mouse (CD1) 2 h	Mouse (CD1) 4 h	Dog 1 h	Dog 2 h	Dog 4 h	Rat 1 h	Rat 2 h	Rat 4 h	Human 1 h	Human 2 h	Human 4 h
Remainder w/o tebuconazole	9.7%	18.1%	21.5%	11.9%	10.8%	4.6%	6.4%	6.8%	10.4%	8.4%	14.2%	14.0%	1.5%	3.1%	1.7%
Hydroxylated tebuconazole	22.6%	10.4%	2.1%	10.9%	7.9%	7.7%	39.6%	33.9%	16.4%	13.8%	2.0%	0.0%	14.9%	2.5%	0.0%
Further oxidised (e.g. keto) compounds	17.3%	20.5%	15.1%	41.7%	34.1%	32.5%	2.2%	5.5%	6.2%	6.8%	3.8%	0.0%	5.2%	7.3%	5.5%
Glcucuronic acid conjugates	21.4%	43.2%	61.4%	31.5%	46.1%	55.3%	14.4%	31.3%	55.5%	66.7%	80.0%	86.1%	62.2%	87.2%	92.8%

Figure 6.1-2. 1 and 5 µM test concentrations

The following observations can be derived from these data:
Test concentrations of 1 and 5 µM

- Glucuronidation was the preferred biochemical reaction in human and rat hepatocytes. Further oxidation to (keto-) compounds were of minor importance.
- In mouse hepatocytes, the further oxidation to (keto-) compounds was additionally important. Compared to human and rat, the glucuronidation was less pronounced.
- In dog hepatocytes, hydroxylation and glucuronidation were the preferred biochemical reactions.

Test concentrations of 10 and 20 µM

- An inhibition of the metabolic capability at higher concentration was observed.
- Glucuronidation was preferred in human and rat hepatocytes. Compared to the 1 µM test concentration, the higher amounts of hydroxylated metabolites in both species indicated possibly a reduced capability for conjugation of these metabolites with glucuronic acid. The further oxidised (keto-) compounds were again of minor importance.
- In mouse hepatocytes, hydroxylation, glucuronidation and further oxidation to (keto-) compounds were on a similar level compared to the 1 and 5 µM test concentrations.
- In dog hepatocytes, the amount of glucuronides was lower and the hydroxylated metabolites higher compared to the 1 µM test concentration. That revealed also a reduced metabolic capability for conjugation of the hydroxylated metabolites with glucuronic acid.

Overall, the results indicate an extensive metabolism of [triazole-UL-^{14}C]tebuconazole in hepatocytes of *all in-vitro* systems leading to a series of phase I and II metabolites.

Conclusion
[Triazole-UL-^{14}C]tebuconazole is intensively metabolised during 2 h incubations in mouse (MH), dog (DH), rat (RH) and human hepatocytes (HH) at test concentrations of 1 and 5 µM. The significant higher amounts of the unchanged test compound at higher test concentrations indicate a possible inhibition of the metabolic capability. The metabolism of tebuconazole in human hepatocytes is best comparable to that of rat. While the principal metabolic reactions (hydroxylation, oxidation, and conjugation) were similar in all hepatocytes incubations the

oxidation and conjugation was most similar in rat and human hepatocytes. Glucuronidation was the most important detoxification pathway in both species. In contrast mouse hepatocytes lead to different oxidised metabolites and showed less conjugation. The lowest coincidences are recognisable in the tests with mouse and dog hepatocytes. All data indicate differences of the metabolic capability for rat and human hepatocytes versus those from dog and mouse.

B.6.1.2.3. ***Protein binding of tebuconazole in plasma of mouse, rat, rabbit, dog and human***

Previous evaluation:	None – submitted for the purpose of renewal (study owned by Bayer Task force)

Study ID	B.6.1.2/03
Study title	Tebuconazole: Investigations on binding to plasma proteins in different species of tebuconazole *in vitro*
Test substance	Tebuconazole (radiolabelled [phenyl-UL-^{14}C]tebuconazole)
Purity (%) **Batch no.**	> 99 (HPLC) KML 12016
Test system	Diluted mouse (NMRI), rat (RccHan:WIST), rabbit (himalayan), dog (beagle) and human plasma
Groups	nominal concentrations 1,000, 10,000 and 100,000 µg/l for each species (3+ pooled)
Dose	Ranging from 948 µg/L to 87,900 µg/L (nominal concentrations 1,000, 10,000 and 100,000 µg/l)
Route	Equilibrium dialysis
Vehicle	PBS buffer
GLP	No
Guideline	n/a
Deviation	n/a
Acceptable	Acceptable

Method
Protein binding of tebuconazole was investigated in plasma of mouse, rat, rabbit, dog and human. The plasma of each species, from at least three individuals and pooled, was diluted as 10% v/v plasma in PBS buffer. Concentrations of the test substance ranged between 948 µg/L and 87,900 µg/L (nominal concentrations ranging from 1,000 µg/L to 100,000 µg/L). The separation of protein bound and free (unbound) fractions of tebuconazole were investigated using the method of equilibrium dialysis across a semipermeable membrane with a pore size of 12 - 14 kDa. The dialysis time was extended to six hours, to ensure that equilibrium was reached in the experiment.

Results
The protein binding was determined in the concentration range from 948 µg/L to 87,900 µg/L (nominal concentrations ranging from 1,000 µg/L to 100,000 µg/L in undiluted plasma) using the method of equilibrium dialysis. The binding of tebuconazole to plasma proteins was moderate in all species investigated. The mean unbound fractions amounted to 3.24% in mouse, 5.09% in man, 5.57% in rabbit, 5.74% in dog and 5.82% in rat. There was no evidence for a concentration dependency in the tested concentration range. There were no relevant species differences.

Table 6.1-19. Overview of plasma protein binding and blood to plasma concentration ratio in different species

Species	**Concentration (range) of tebuconazole for protein binding assay [µg/l]**	**fraction unbound ($f_{u)}$ [%]**
Human (f)	974 - 87900	5.09
Wistar rat (f)	970 - 86630	5.82
Beagle dog (f)	949 - 86590	5.74
Himalayan rabbit (f)	948 - 87480	5.57
NMRI mouse (f)	953 - 87010	3.24

Conclusion
The protein binding of [phenyl-UL-^{14}C]tebuconazole was investigated *in vitro* in diluted plasma of mouse, rat, rabbit, dog and human. The unbound fraction of tebuconazole in plasma was only about 5 % in all species indicating that the systemically available concentration of unbound tebuconazole in toxicological studies *in vivo* is likely much lower than indicated by the given dose. There was no evidence for a concentration dependency in the tested concentration range and there were no relevant species differences.

B.6.1.2.4. ***Overall summary in vitro studies***

Two *in-vitro* interspecies comparative metabolism studies were conducted with the parent substance tebuconazole radiolabelled in the triazole moiety using either mouse, rat and human liver S9 fractions, or mouse, dog, rat, and human hepatocytes. Additionally, plasma protein binding of [phenyl-UL-^{14}C] tebuconazole was investigated in plasma of mouse, rat, rabbit, dog and human. These *in vitro* studies were submitted for the purposes of renewal by the Bayer Task Force. No *in-vitro* interspecies comparative metabolism studies were submitted by the EU Tebuconazole Task Force.

The comparative *in vitro* metabolism study using mouse, rat and human liver S9 fractions was conducted to compare the metabolic pattern in the different species and to demonstrate that the laboratory animals serve as suitable animal models for the metabolism in human.

The incubations in the presence of S9 liver homogenates showed metabolisation of tebuconazole with the highest transformation in rat liver homogenates. The biotransformation of tebuconazole was generally higher after incubation at a concentration of 1 µM compared to 10 µM indicating inhibition of the metabolic capacity by higher concentrations of tebuconazole. One major metabolite was formed similarly in all test systems. Comparison of the metabolic profiles showed that no unique human metabolite had been formed.

As in the rat a higher metabolic transformation of tebuconazole was observed, a further *in vitro* metabolism study was conducted to compare the metabolic profile of tebuconazole in intact primary cells (hepatocytes) isolated from mouse, dog, rat and human liver, since this organ is the preferred site for metabolism in animals.

[Triazole-UL-^{14}C]tebuconazole was extensively metabolised during 2 h incubations in mouse (MH), dog (DH), rat (RH) and human hepatocytes (HH) at test concentrations of 1 and 5 µM compared to 10 and 20 µM. The significant higher amounts of the unchanged test compound at higher test concentrations of all *in vitro* metabolism studies indicate inhibition of the metabolic capability. Rat and human hepatocytes showed the highest metabolic transformation of tebuconazole and the most similar metabolic pattern compared to the other species. While the principal metabolic reactions (hydroxylation, oxidation, and conjugation) were similar in all hepatocytes incubations, the kind of oxidation and conjugation was most similar again in rat and human hepatocytes. Glucuronidation was the most important detoxification pathway in both species. In contrast mouse hepatocytes lead to different oxidised metabolites and other and less conjugation. The metabolism of tebuconazole in human hepatocytes is best comparable to that of the rat.

The protein binding of [phenyl-UL-^{14}C]tebuconazole was investigated *in vitro* in diluted plasma of mouse, rat, rabbit, dog and human. The unbound fraction of tebuconazole in plasma was about 5 % in all species indicating that the systemically available concentration of unbound tebuconazole in toxicological studies *in vivo* is likely to be much lower than indicated by the given dose. There was no evidence for a concentration dependency of protein binding in the tested concentration range and there were no relevant species differences observed.

B.6.1.3. Overall summary on absorption, distribution, excretion and metabolism (toxicokinetics)

Three toxicokinetic studies were evaluated in the original DAR and are reproduced in this RAR; these utilised [phenyl-UL-^{14}C] tebuconazole or [triazol-3-5-^{14}C]tebuconazole administered in single (low- and high-dose) or repeated (low) oral doses. Three publications of relevance to toxicokinetics have also been considered. In addition, two new *in vitro* interspecies comparative metabolism studies, and a new protein binding study have been provided by the Bayer Task Force (TF) for the purpose of renewal. No *in vitro* interspecies comparative metabolism studies were submitted by the EU Tebuconazole Task Force (TF).

The following key conclusions were obtained from the evaluation of the toxicokinetic information:

- A correction to take into account oral absorption is not required for the calculation of the systemic AOEL

/ AAOEL.

- Dermal absorption from the representative product(s) are as follows:
  - Folicur (EW 250): 0.6 % (concentrate 250 g/L), 11 % (dilution 2.5 g/L) and 19 % (dilution 0.1 g/L).
  - Redigo Pro (FS 170): 1 % (concentrate 20 g/L), 4 % (dilution 11.43 g/L) and 10 % (dilution 0.8 g/L).
  - CA 2368 (EW 250): 0.3 % (concentrate 250 g/L), 19 % (dilution 0.625 g/L) and 24 % (dilution 0.1 g/L).
  - SIP 40957 (EW 250): 0.9 % (concentrate 250 g/L), 18 % (dilution 0.42 g/L) and 33 % (dilution 0.1 g/L).
- Inhalation exposure is assumed to be 100 % (based on high oral absorption).
- The data requirements of Regulation 283/2013 have been met.

Type of study	Dose levels (mg/kg b.w.) or Concentration	Animal species, strain; sex, test system	Substance	Findings	References
ADME study - single dose study	2 and 20 mg/kg bw	Rats, Wistar (BOR:WISW), males and females	Tebuconazole (phenyl-UL-^{14}C) (HWG 1608)	Almost complete absorption of tebuconazole after oral administration. A large part of the elimination of tebuconazole was via the bile (91 % within 48 h; 50 % of the total biliary excretion was eliminated after 2.5 h and 90 % after 7 h, indicating a significant first pass effect).	B.6.1.1.1/01
Whole-body autoradio-graphic distribution	20.0 mg/kg bw	Male Wistar (BOR:WISW) rats	Tebuconazole (phenyl-UL-^{14}C) (HWG 1608)	The study showed an even distribution of tebuconazole. 1 hour after administration radioactivity was detectable in all body tissues with the exception of the compact bone substance.	B.6.1.1.2/01
Metabolism study	Single dose: 2 or 20 mg/kg bw; in some groups pre-treatment with 2 mg/kg bw of non-radioactive test substance	Male and female Wistar (BOR:WISW) rats	Tebuconazole (phenyl-UL-^{14}C & triazol-3,5-^{14}C) (HWG 1608)	Tebuconazole was efficiently metabolised as hardly any unchanged parent compound was found in the excreta 72 h after administration.	B.6.1.1.3/01
In-vitro metabolism study	1 and 10 µM	Mouse; liver S9 fractions Rat and human; liver S9 fractions	Tebuconazole (triazol-UL-^{14}C)	Slight or no biotransformation after incubation with mouse liver S9 fraction. High or moderate biotransformation after incubation with rat and human liver S9 fraction. No human unique metabolites were detected.	B.6.1.1.3/01
In-vitro metabolism	1, 5, 10 and 20 µM	Dog, human, rat, mouse;	Tebuconazole (triazol-UL-	Intense biotransformation after 2 h incubations at 1	B.6.1.2/02

Type of study	Dose levels (mg/kg b.w.) or Concentration	Animal species, strain; sex, test system	Substance	Findings	References
study		hepatocytes	^{14}C)	and 5 µM. The principal metabolic reactions were hydroxylation, oxidation, and conjugation with glucuronic acid as the preferred molecule for the conjugation. No human unique metabolites were detected.	
Protein binding study	0.32 – 32 µM	Dog, human, rat, rabbit, mouse; plasma	Tebuconazole (phenyl-UL-^{14}C)	The binding of tebuconazole to plasma proteins was moderate in all species investigated without evidence for a concentration dependency.	B.6.1.2/03

The absorption of tebuconazole from the gastro-intestinal tract of the rat is rapid and complete based on urinary (7.4 %) and biliary (90.9 %) excretion by the cholecystotomized animals within 48 hours. A figure of > 98 % (100 % for the purpose of AOEL derivation) of the oral dose was therefore obtained for the degree of oral absorption. Peak relative concentration in the blood plasma was found from 20 to 100 minutes after administration (B.6.1.1.1/01). Absorption of tebuconazole was slower at the high dose and repeated pre-treatment with the non-labelled test substance had no impact on absorption. Sex-dependent differences were apparent. There are no kinetic data for the inhalation route; however, considering the high oral absorption, it can be assumed that inhalation absorption is also 100 %. Dermal absorption of tebuconazole is product-specific and is addressed in the CP-B6 documents: Folicur (EW 250): 0.6 % (concentrate 250 g/L), 11 % (dilution 2.5 g/L) and 19 % (dilution 0.1 g/L). Redigo Pro (FS 170): 1 % (concentrate 20 g/L), 4 % (dilution 11.43 g/L) and 10 % (dilution 0.8 g/L). CA 2368 (EW 250): 0.3 % (concentrate 250 g/L), 19 % (dilution 0.625 g/L) and 24 % (dilution 0.1 g/L). SIP 40957 (EW 250): 0.9 % (concentrate 250 g/L), 18 % (dilution 0.42 g/L) and 33 % (dilution 0.1 g/L).

The distribution in the body was studied in a whole-body autoradiographic study (B.6.1.1.2/01). One hour after oral administration tebuconazole was rapidly and evenly distributed into organs and tissues, with the exception of compact bone substance. Highest levels were found in the liver. Tebuconazole did not accumulate in any organ or tissue after oral administration: radiolabelled residues in tissues and organs were low at termination, but generally higher in male rats compared with females.

The metabolism study in rats revealed that tebuconazole is efficiently metabolised as hardly any unchanged parent compound (0.5 - 2.2 % administered dose) is found in the excreta 72 h after administration. Ten compounds, excluding the parent compound, were identified in urine and faeces. M03 (tebuconazole-1-hydroxy) and M06 (tebuconazole-carboxylic acid) were major metabolites in all test groups with a slight tendency towards higher amounts in females. M26 (1,2,4-triazole) was also identified in the rat but at levels up to a max of 5 %. The same metabolites (TEB-OH and TEB-COOH) were identified, both as free molecules and as glucuronide conjugates, as the main metabolites of tebuconazole in the urine of vineyard workers exposed to tebuconazole.

Distinct sex differences were seen in the metabolic pattern of tebuconazole which mainly involves oxidations as phase 1- reactions, resulting in hydroxy, carboxy, triol and ketoacid metabolites and the phase 2 - conjugates were glucuronides and sulphates. Furthermore, the break-down product 1,2,4-triazole amounted to 5 % in the urine of the male and 1.6 % in that of the female rat (B.6.1.1.3/01). Neither the dose level nor the repeated pre-treatment showed a significant influence on the metabolic pattern in any of the dose groups.

A comparative *in-vitro* metabolism study using mouse, rat and human liver S9 fractions showed metabolism of tebuconazole with the highest transformation in rat liver homogenates. One major metabolite was formed similarly in all test systems. Comparison of the metabolic profiles showed that no unique human metabolite had been formed.
In a further comparative *in vitro* metabolism study on rat and human cells showed the highest metabolic

transformation of tebuconazole and the most similar metabolic pattern compared to the other species. While the principal metabolic reactions (hydroxylation, oxidation, and conjugation) were similar in all hepatocytes, the kind of oxidation and conjugation was most similar again in rat and human hepatocytes. Glucuronidation was the most important detoxification pathway in both species. In contrast, incubation with mouse hepatocytes led to different oxidised metabolites and less conjugation. These *in vitro* studies therefore indicate that the metabolism of tebuconazole by human hepatocytes is broadly comparable to that by rat hepatocytes.

The protein binding of [phenyl-UL-^{14}C] tebuconazole was investigated *in-vitro* in diluted plasma of mouse, rat, rabbit, dog and human. The unbound fraction of tebuconazole in plasma was about 5 % in all species indicating that the systemically available concentration of unbound tebuconazole in toxicological studies *in-vivo* is likely to be much lower than indicated by the given dose.

The excretion of the radioactivity was a fast and complete process which mainly took place via faeces as 62 – 82 % of the administered dose was eliminated by the route, whereas elimination in urine amounted to about 15 – 33 % (B.6.1.1.1/01) in intact animals. For cannulated animals 92.2 % of the administered dose were eliminated by the biliary and faecal route and 7.4 % via urine (B.6.1.1.1/01). Sex-dependent differences were apparent including generally slower clearance of tebuconazole in male rats at all doses. Biliary and faecal elimination was greater in males than in females with correspondingly lower excretion via urine. The amount excreted was not related to the administered dose. The results indicate that enterohepatic recirculation occurs in intact animals. Less than 1 % of the administered dose was recovered in the tissues two to three days after administration, with the liver containing most of the tissue residues. Male animals in all groups had higher residue levels than females. Only a very small amount of radioactivity (0.032 %) was detected in the exhaled air within 3 days of oral administration of 20 mg/kg bw.

The proposed metabolic partway of tebuconazole is shown in Figure 6.1-3.

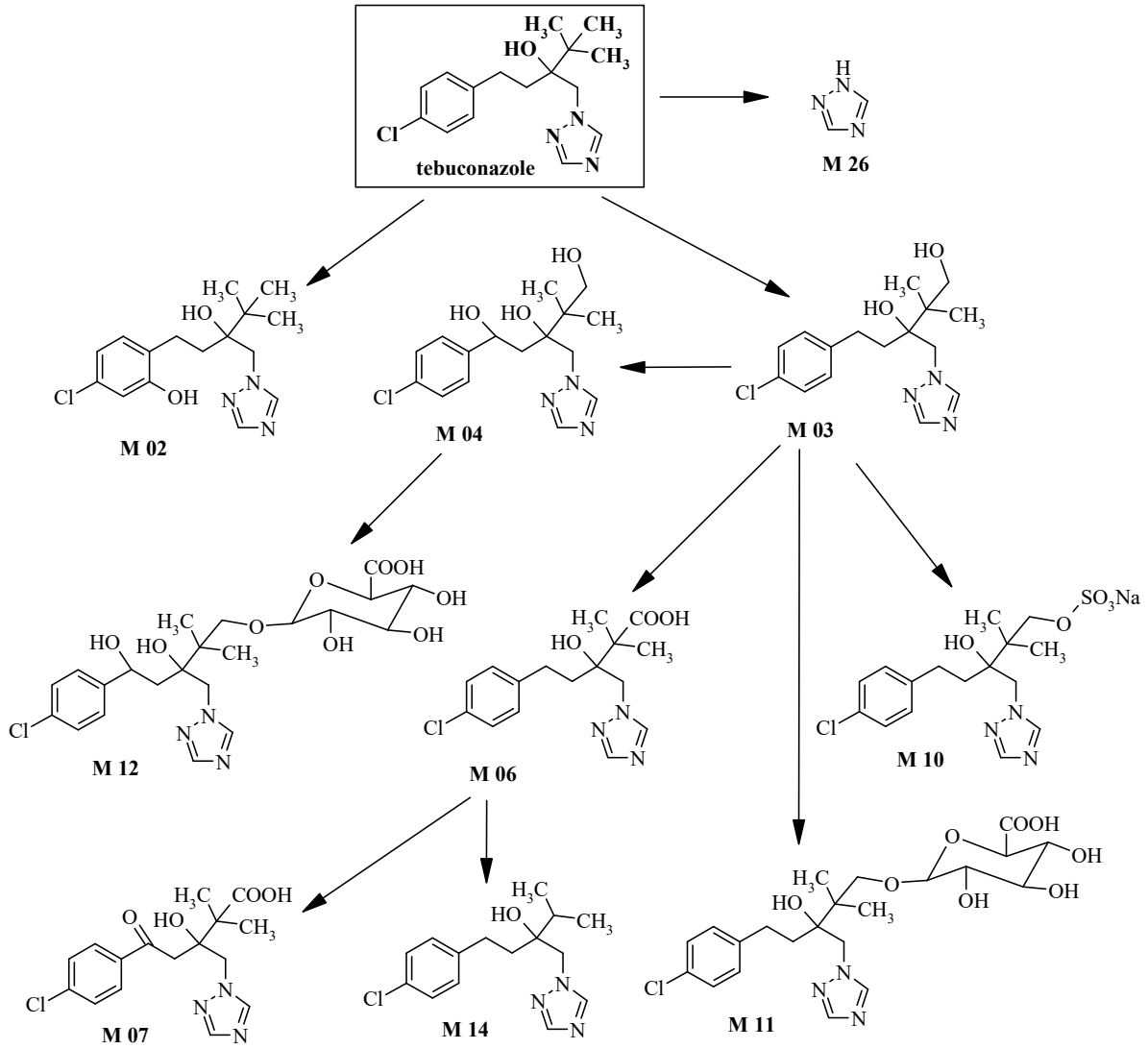


Figure 6.1-3. Proposed metabolic pathway of tebuconazole in the rat

M02:	HWG 1608-o-hydroxy	*M10*:	HWG 1608-1-hydroxysulphate
M03:	HWG 1608-1-hydroxy	*M11*:	HWG 1608-1-OH-glucuronide
M04:	HWG 1608-1,5-dihydroxy	*M12*:	HWG 1608-1,5-di-OH-glucuronide
M06:	HWG 1608-carboxylic acid	*M14*:	HWG 1608-desmethyl
M07:	HWG 1608-ketocarboxylic acid	*M26*:	1,2,4-triazole

B.6.2. ACUTE TOXICITY

The acute toxicity of tebuconazole was investigated in multiple studies conducted via the oral, dermal and inhalation routes. Studies of skin irritancy, eye irritancy and skin sensitisation were also conducted. The available studies, owned by Bayer Task Force (TF), were evaluated in the original DAR (2006), peer reviewed at EU level (EFSA Conclusion, 2008), and were considered acceptable.

In addition, the Bayer TF have provided a new study investigating the acute toxicity via the inhalation route. The EU Tebuconazole Task Force have provided new data investigating acute dermal and inhalation toxicity, plus studies of skin irritancy, eye irritancy and skin sensitisation. No phototoxicity studies have been provided as testing is not triggered according to data requirements in Reg 283/2013.

EU agreed acute toxicity endpoints for Tebuconazole (EFSA Scientific report (2008), 176, p53)		Classification (1272/2008)
Rat LD50 oral	1700 mg/kg bw (f)	Acute Tox 4, H302
Rat LD50 dermal	> 2000 mg/kg bw	-
Rat LC50 inhalation	> 5.093 mg/L (nose only 4 h)	-
Skin irritation	Non-irritant	-
Eye irritation	Non-irritant	-
Skin sensitisation	Non-sensitiser (M&K test)	-

B.6.2.1. Oral

The acute oral toxicity of tebuconazole has been investigated in a total of six studies in multiple species (3 in rats, 2 in mice and 1 in rabbits). The available studies, owned by the Bayer TF, were evaluated in the original DAR (2006), peer reviewed at EU level (EFSA Conclusion, 2008) and considered to be acceptable. These are re-summarised below. The results of these studies show that the acute oral LD_{50} of tebuconazole is 1700 mg/kg bw in rats. Classification for acute oral toxicity, category 4, H302, is required under CLP Regulation (EC) No. 1272/2008. This is consistent with the harmonised classification of tebuconazole.

No new acute oral studies have been provided for the purpose of renewal by the Bayer TF. No acute oral studies have been provided by the EU Tebuconazole TF.

B.6.2.1.1. *Acute oral toxicity in rats*

a)

Previous evaluation:	In tebuconazole DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.2.1.1/01
Study title	HWG 1608 Technical – Acute oral toxicity study on rats
Test substance	(HWG 1608) Tebuconazole
Purity (%) **Batch no.**	98.0 (technical grade) 816096181
Test animals	Male and Female Sprague-Dawley rat (Crj:CD)
Groups	5 males and 5 females/dose
Dose	Males: 1600, 2300, 3000, 3900 and 5000 mg/kg body weight. Females: 730, 950, 1230, 1600, 2300, 3000, 3900 and 5000 mg/kg body weight
Route	Oral by gavage (fasted animals)
Vehicle	Suspension in polyethylene glycol 400
GLP	Yes
Guideline	Comparable to OECD Guideline 401.
Deviation	The following deviations from the OECD-Guideline 401 (1987) occurred: None
Acceptable	Acceptable
LD_{50}	1700 mg/kg bw

Methods
Tebuconazole, formulated in polyethylene glycol 400, was administered oral by gavage to groups of 5 male and 5 female rats in a single dose at dose levels 1600, 2300, 3000, 3900 and 5000 mg/kg body weight (males) and 730, 950, 1230, 1600, 2300, 3000, 3900 and 5000 mg/kg body weight (females). The post-treatment observation period lasted 14 days. Clinical signs were recorded at the day of administration and further once a day. Body weight was recorded at the day of administration and further on a weekly basis. Necropsy was performed at the end of the post-treatment period, and all animals were subjected to a gross pathology examination. Animals that died under the observation period were also subjected to a gross pathology examination. A validated method of analysis for single exposure gavage studies is not required.

Results
Mortality
A total of 7 males (from 3000-5000 mg/kg bw) and 22 females (from 950-5000 mg/kg bw) died (Table 6.2-1.).

Body weight
There were no treatment related effects on body weight and body weight development in male and female rats at the end of the study.

Clinical signs
Main symptoms were sedation, abnormal gait, paralytic gait and emaciation which were observed in male and females.

Pathology
At termination abnormal findings in the liver (yellow-white patchy areas) and the testis (atrophy) for males were observed. Changes in the urinary bladder (reddish content), the adrenals (redness and hypertrophy) and in the trachea (retention of foamy fluid) were observed in animals (male and female) that died during the observation period.

Table 6.2-1. Acute oral toxicity

Dose [mg/kg bw]	Toxicological results			Duration of clinical signs	Time of death
	Dead	Toxic signs	Used		
Males					
1600	0	4	5	20min – 1h	-
2300	0	5	5	3d – 5d	-
3000	1	4	5	2d – 5d	3d
3900	2	5	5	20min – 5d	3d – 4d
5000	4	5	5	2h – 7d	4d – 6d
LD_{50} value: 4000 mg/kg bw (95% confidence limit 3300 – 5800 mg/kg bw)					
Females					
730	0	0	5	-	-
950	1	3	5	5h – 2d	2d
1230	0	3	5	5h – 9d	-
1600	2	4	5	20min – 6d	1d – 3d
2300	5	5	5	3d	4d – 8d
3000	4	5	5	2d – 7d	4d – 5d
3900	5	5	5	30min	3d – 8d
5000	5	5	5	2h	3d – 5d
LD_{50} value: 1700 mg/kg bw (95% confidence limit 1400-2200 mg/kg bw)					

Conclusion
This acute oral toxicity study of tebuconazole was investigated in accordance with GLP and OECD/EU guidelines. The test substance showed slight to moderate oral toxicity in rats. The observed clinical symptoms were considered to be similar to those of central nervous system depressants such as anaesthetic agents. There was no sex difference in observed symptoms, the onset and the disappearance time of the symptoms and death, but tebuconazole was more acutely toxic to female rats than male rats. Abnormal findings in the liver were considered to be due to tebuconazole because these were observed dose-dependently. Because the findings in the urinary bladder, the adrenals and the trachea were observed only in a few animals, these were not considered to be due to tebuconazole administration.

The following LD_{50} values were established (calculated by method of Bliss).
LD_{50} for male rats: 4000 mg/kg bw (3300 – 5800 mg/kg bw)
LD_{50} for female rats: 1700 mg/kg bw (1400-2200 mg/kg bw)

Under the conditions of the study with rats, tebuconazole is classified as category 4 for acute oral toxicity (H302) (female rats LD50 > 300 and < 2000 mg/kg bw) according to Regulation (EC) No. 1272/2008.

b)

Previous evaluation:	In tebuconazole DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.2.1.1/02
Study title	HWG 1608 and KWG 0519 / HWG 1608 and KUE 13032c - Combination toxicity study
Test substance	(HWG 1608) Tebuconazole
Purity (%) **Batch no.**	94.7 16002/86
Test animals	Male Wistar rats/Bor:WISW (SPF-Cpb)
Groups	5 males at single dose
Dose	5000 mg/kg body weight.
Route	Oral by gavage (fasted animals)
Vehicle	Cremophor EL/demineralized water (2 %)
GLP	No – at the time the study was performed GLP was not compulsory
Guideline	Comparable to OECD Guideline 401.
Deviation	The following deviations from the OECD-Guideline 401 (1987) occurred: - None
Acceptable	Acceptable
LD_{50}	> 5000 mg/kg

Methods
Tebuconazole, formulated in Cremophor EL/demineralized water (2 %), was administered oral by gavage to 5 male rats in a single dose at dose 5000 mg/kg body weight. The post-treatment observation period lasted 14 days. Clinical signs were recorded at the day of administration and further once a day. Body weight was recorded at the day of administration and further on a weekly basis. Necropsy was performed at the end of the post-treatment period, and all animals were subjected to a gross pathology examination. Animals that died under the observation period were also subjected to a gross pathology examination. A validated method of analysis for single exposure gavage studies is not required.

Results
Mortality
One rat died at day six.

Body weight
There were no treatment related effects on body weight and body weight development at the end of the study.

Clinical signs
Bristled fur, apathy, reduced motility, spastic gait, staggering, dyspnoea, salivation, diarrhoea was observed.

Pathology
At termination abnormal findings in the liver (thin yellowish layer), lungs (patchy, dark spots) and scar like changes were observed.

Conclusion
This acute oral toxicity study of tebuconazole was investigated in accordance with OECD/EU guidelines. The test substance showed no acute oral toxicity in rats.

The following LD_{50} value was established (calculated by method of Bliss): LD_{50}: > 5000 mg/kg bw. On the basis of the findings in this study in rats, no classification for acute oral toxicity is required (LD_{50} > 2000 mg/kg bw) according to Regulation (EC) No. 1272/2008.

c)

Previous evaluation:	In tebuconazole DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.2.1.1/03
Study title	HWG 1608 - Study for acute toxicity

Test substance	(HWG 1608) Tebuconazole
Purity (%) **Batch no.**	97.1 16001/83
Test animals	Male and female Wistar rats/Bor:WISW (SPF-Cpb)
Groups	5 rats/sex/dose
Dose	Fasted: 1000, 2500, 4500 and 5000 mg/kg bw (male) 1000, 2500, 3150, 3550 and 5000 mg/kg bw (female Non-fasted: 500, 1000, 3550, 3750, 4000, 5000 mg/kg bw (male) 500, 1000, 2500, 3550, 4250, 4500 mg/kg bw (female)
Route	Oral by gavage
Vehicle	Cremophor EL/water
GLP	No
Guideline	OECD Guideline 401
Deviation	The following deviations from OECD-Guideline 401 (1987) occurred: - None
Acceptable	Acceptable
LD_{50}	3352 mg/kg bw

Method
Tebuconazole, formulated in Cremophor EL/water, was administered oral by gavage to groups of 5 to 10 male and 5 female Wistar rats in a single dose (10 mL/kg bw) at dose levels 1000, 2500, 4500 and 5000 mg/kg bw (fasted male) or 1000, 2500, 3150, 3550 and 5000 mg/kg bw (fasted female) and 500, 1000, 3550, 3750, 4000, 5000 mg/kg bw (non-fasted male) or 500, 1000, 2500, 3550, 4250, 4500 mg/kg bw (non-fasted female).The post-treatment observation period lasted 14 days. Clinical signs were recorded at the day of administration and further once a day. Body weight was recorded at the day of administration and further on a weekly basis. Necropsy was performed at the end of the post-treatment period, and all animals were subjected to a gross pathology examination. Animals that died under the observation period were also subjected to a gross pathology examination. A validated method of analysis for single exposure gavage studies is not required.

Results
Mortality
A total of 2 fasted males (5000 mg/kg bw) and 7 fasted females (from 3150-5000 mg/kg bw) and a total of 9 non-fasted males (from 3750-5000 mg/kg bw) and 12 non-fasted females (from 2500-4500 mg/kg bw) died.
(Table 6.2-2.)

Body weight
Weight loss was observed in the first week of the post-treatment observation period (fasted male: 4500, 5000 mg/kg bw, fasted female: 3550, 5000 mg/kg bw, non-fasted male: 3750, 4000, 5000 mg/kg bw, non-fasted female: 2500, 3550, 4250 mg/kg bw) but normalized at the end of the period.

Clinical signs
Main symptoms seen were behavioural disturbances, breathing disturbances, motility disturbances, staggering, spastic gait, sternal, lateral recumbency, loss of hair, cramped posture, increased urine excretion and poor reflexes which were observed in males and females.

Pathology
Animals which died during post-treatment observation period: lungs (spotted, distended), liver (patchy, pale, lobulation, enlarged), glandular stomach (reddened). Animals sacrificed at termination: no treatment-related findings.

Table 6.2-2. Acute oral toxicity

Dose [mg/kg bw]	Toxicological results*			Duration of clinical signs	Time of death
	Dead	Toxic signs	Used		
Fasted male rats					
1000	0	0	5	-	-
2500	0	5	5	4h – 10d	-

Dose [mg/kg bw]	Toxicological results*			Duration of clinical signs	Time of death
	Dead	Toxic signs	Used		
4500	0	5	5	5h – 12d	-
5000	2	10	10	4h – 14d	7– 11d
LD_{50}: > 5000 mg/kg bw					
Fasted female rats					
1000	0/0/5	0	5	-	-
2500	0	5	5	4h – 9d	-
3150	1	5	5	4h – 8d	5d
3550	2	5	5	4h – 10d	4-8d
5000	4	5	5	4h – 10d	2-8d
LD_{50}: 3933 mg/kg bw (95% confidence interval 3316.1 – 5665.2 mg/kg bw)					
Non-fasted male rats					
500	0	0	0	-	-
1000	0	5	5	4h-3d	-
3550	0	10	10	4h-10d	-
3750	3	10	10	4h-14d	4-6d
4000	2	5	5	4h-12d	4-5d
5000	4	5	5	4h-10d	5-7d
LD_{50}: 4264 mg/kg bw (95% confidence interval 3952.3 – 5330.2 mg/kg bw)					
Non-fasted female rats					
500	0	0	5	-	-
1000	0	5	5	1d – 6d	-
2500	1	5	5	5d – 10d	6d
3550	2	5	5	4h-9d	4-7d
4250	4	5	5	2d-11d	5-7d
4500	5	5	5	4h-9d	3-9d
LD_{50}: 3352 mg/kg bw (95% confidence interval 2341.4 – 3977.5 mg/kg bw)					

* First number = number of dead animals, second number = number of animals with toxic signs, third number = number of animals used

Conclusion
This acute oral toxicity study of tebuconazole was investigated in accordance with GLP and OECD/EU guidelines. Tebuconazole was of low toxicity to fasted and non-fasted male and female rats after acute oral administration.

The following LD_{50} values were established:
LD_{50} for male rats: LD_{50} > 5000 mg/kg bw (fasted), LD_{50}: 4264 mg/kg bw (non-fasted).
LD_{50} for female rats: LD_{50}: 3933 mg/kg bw (fasted), LD_{50}: 3352 mg/kg bw (non-fasted).

Based on the findings of this study in rats, no classification for acute oral toxicity is required (LD_{50} >2000 mg/kg bw) according to Regulation (EC) No. 1272/2008.

B.6.2.1.2. *Acute oral toxicity in mice*

a)

Previous evaluation:	In tebuconazole DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.2.1.2/01
Study title	HWG 1608 technical - Acute oral toxicity study on mice
Test substance	(HWG 1608) Tebuconazole
Purity (%) **Batch no.**	98.0 (technical grade) 816096181
Test animals	Male and female ICR (Crj:CD-1) mice
Groups	5 mice/sex/dose
Dose	Males: 1600, 2300, 3000, 3900 and 5000 mg/kg body weight.

	Females: 3000, 3900 and 5000 mg/kg body weight.
Route	Oral by gavage (fasted animals)
Vehicle	polyethylene glycol 400
GLP	Yes
Guideline	Comparable to OECD Guideline 401.
Deviation	The following deviations from the OECD-Guideline 401 (1987) occurred: - None
Acceptable	Acceptable – supplementary
LD_{50}	2800 mg/kg

Methods
Tebuconazole, formulated in polyethylene glycol 400, was administered oral by gavage to groups of 5 male and 5 female mice in a single dose at dose levels 1600, 2300, 3000, 3900 and 5000 mg/kg body weight (males) and 3000, 3900 and 5000 mg/kg body weight (females).The post-treatment observation period lasted 14 days. Clinical signs were recorded at the day of administration and further once a day. Body weight was recorded at the day of administration and further on a weekly basis. Necropsy was performed at the end of the post-treatment period, and all animals were subjected to a gross pathology examination. Animals that died under the observation period were also subjected to a gross pathology examination. A validated method of analysis for single exposure gavage studies is not required.

Results
Mortality
A total of 13 males (from 1600-5000 mg/kg bw) and 4 females (from 3900-5000 mg/kg bw) died (Table 6.2-3.).

Body weight
There were no treatment related effects on body weight and body weight development in male and female rats at the end of the study.

Clinical signs
Main symptoms were sedation, abnormal gait, paralytic gait and hypnosis which were observed in male and females.

Pathology
At termination abnormal findings in the liver (yellow-white patchy areas) for males were observed. Changes in the digestive system (mucosal redness, dark reddish brown focus in the stomach, dilated lumen, yellowish contents and mucosal redness in the small intestine), lungs (dark reddish brown) and testis (atrophy) were observed in animals that died during the observation period.

Table 6.2-3. Acute oral toxicity (rat)

Dose [mg/kg bw]	**Toxicological results**			**Duration of clinical signs**	**Time of death**
	Dead	Toxic signs	Used		
Males					
1600	1	5	5	5’ – 2h	2d
2300	3	5	5	4’ – 1d	1d – 3d
3000	1	5	5	9’ – 2d	8d – 8d
3900	3	4	5	3’ – 5d	2d
5000	5	5	5	6’	1d – 3d
LD_{50}: 2800 mg/kg bw (95% confidence interval 1200 – 4900 mg/kg bw)					
Females					
3000	0	2	5	7’ – 3h	--
3900	2	5	5	4’ – 3h	2d – 3d
5000	2	4	5	1’ – 5d	2d
LD_{50}: 5200 mg/kg bw					

Conclusion
This acute oral toxicity study of tebuconazole was investigated in accordance with GLP and OECD/EU guidelines.

The test substance showed slight oral toxicity in mice. The observed clinical symptoms were considered to be similar to those of central nervous system depressant such as anaesthetic agent. There was no sex difference in observed symptoms, the onset and the disappearance time of the symptoms and death, but tebuconazole was more acutely toxic to male mice than female mice. Abnormal findings in the digestive system were considered to be due to tebuconazole.

The following LD50 values were established.
LD_{50} for male mice: LD_{50}: 2800 mg/kg bw (95% confidence interval 1200 – 4900 mg/kg bw).
LD_{50} for female mice: LD_{50}: 5200 mg/kg bw.

Based upon the findings of this study in mice, no classification for acute oral toxicity is required (LD_{50} >2000 mg/kg bw) according to Regulation (EC) No. 1272/2008.

b)

Previous evaluation:	In tebuconazole DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.2.1.2/02
Study title	HWG 1608 - Study for acute toxicity
Test substance	(HWG 1608) Tebuconazole
Purity (%) **Batch no.**	97.1 16001/83
Test animals	Male and female NMRI mice
Groups	5 mice/sex/dose
Dose	100, 500, 1000, 1800, 2500, 3150 and 3550 mg/kg bw (male) 500, 1000, 1800, 2500, 3550 and 5000 mg/kg bw (female)
Route	Oral by gavage (fasted animals)
Vehicle	Cremophor EL/water
GLP	No
Guideline	OECD Guideline 401.
Deviation	The following deviations from the OECD-Guideline 401 (1987) occurred: - None
Acceptable	Acceptable – supplementary
LD_{50}	1615 mg/kg

Method
Tebuconazole, formulated in Cremophor EL/water, was administered oral by gavage to groups of 5 male and 5 female mice in a single dose (10 mL/kg bw) at dose levels 100, 500, 1000, 1800, 2500, 3150 and 3550 mg/kg bw (male) and 500, 1000, 1800, 2500, 3550 and 5000 mg/kg bw (female).The post-treatment observation period lasted 14 days. Clinical signs were recorded at the day of administration and further once a day. Body weight was recorded at the day of administration and further on a weekly basis. Necropsy was performed at the end of the post-treatment period, and all animals were subjected to a gross pathology examination. Animals that died under the observation period were also subjected to a gross pathology examination. A validated method of analysis for single exposure gavage studies is not required.

Results
Mortality
A total of 17 fasted males mice (1000-3550 mg/kg bw) and 10 fasted females mice (from 1800-5000 mg/kg bw) (Table 6.2-4.) died.

Body weight
Weight loss was observed in the first week of the post-treatment observation period (female: 5000 mg/kg bw) but normalized at the end of the period.

Clinical signs
Main symptoms seen were behavioural disturbances, breathing disturbances, motility disturbances, staggering, spastic gait, sternal, lateral recumbency, poor reflexes in males and females.

Pathology
Animals which died during post-treatment observation period: lungs spotted, distended; liver patchy, pale, lobulation, enlarged; spleen patchy; kidney patchy; glandular stomach reddened. Animals sacrificed at termination: no treatment-related findings.

Table 6.2-4. Acute oral toxicity (mice)

Dose [mg/kg bw]	Toxicological results			Duration of clinical signs	Time of death
	Dead	Toxic signs	Used		
Fasted male mice					
100	0	0	5	-	-
500	0	5	5	2h – 1d	-
1000	1	5	5	1h – 6d	2d
1800	3	5	5	1h – 6d	1– 3d
2500	4	5	5	33`-8d	1– 2d
3150	4	5	5	31`-8d	1d
3550	5	5	5	23`-8d	1– 3d
LD_{50}: 1615 mg/kg bw					
Fasted female mice					
500	0	0	5	-	-
1000	0	5	5	4h – 9d	-
1800	1	5	5	4h – 8d	1d
2500	2	5	5	4h – 10d	1-5d
3550	3	5	5	4h – 10d	1d
5000	4	5	5		1-9d
LD_{50}: 3023 mg/kg bw					

Conclusion
This acute oral toxicity study of tebuconazole was investigated in accordance with GLP and OECD/EU guidelines. Tebuconazole was slightly toxic to fasted male and female mice after acute oral administration.

The following LD_{50} values were established.
LD_{50} for male mice: 1615 mg/kg bw
LD_{50} for female mice: 3023 mg/kg bw

Under the conditions of the study with mice tebuconazole requires classification with category 4 for acute oral toxicity (H302) (male mice LD_{50} > 300 and < 2000 mg/kg bw) according to Regulation (EC) No. 1272/2008.

B.6.2.1.3. *Acute oral toxicity in rabbits*

a)

Previous evaluation:	In tebuconazole DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.2.1.3/01
Study title	HWG 1608 - Study for acute toxicity
Test substance	(HWG 1608) Tebuconazole
Purity (%) **Batch no.**	97.1 16001/83
Test animals	Male and female Albino rabbits (HC:NZW)
Groups	3 rabbits/sex/dose
Dose	500 and 1000 mg/kg bw (male and female)
Route	Oral by gavage (fasted animals)
Vehicle	Cremophor EL/water
GLP	No

Guideline	OECD Guideline 401.
Deviation	The following deviations from the OECD-Guideline 401 (1987) occurred: none.
Acceptable	Acceptable – supplementary
LD_{50}	>1000 mg/kg

Method
Tebuconazole, formulated in Cremophor EL/water, was administered oral by gavage to groups of 3 male and 3 female rabbits (fasted for approx. 16 hrs) in a single dose (0.5 mL/kg bw) at dose levels 500 and 1000 mg/kg bw. The post-treatment observation period lasted 14 days. Clinical signs were recorded at the day of administration and further once a day. Body weight was recorded at the day of administration and further on a weekly basis. Necropsy was performed at the end of the post-treatment period, and all animals were subjected to a gross pathology examination. Animals that died under the observation period were also subjected to a gross pathology examination. A validated method of analysis for single exposure gavage studies is not required.

Results
Mortality
No mortality was observed during the study.

Body weight
There were no treatment related effects on body weight.

Clinical signs
A general loss of appetite was observed

Pathology
In animals sacrificed at termination the following were observed: lung slightly distended, spotted, kidney slightly patchy.

Table 6.2-5. Acute oral toxicity (rabbit)

Dose [mg/kg bw]	Toxicological results			Duration of clinical signs	Time of death
	Dead	Toxic signs	Used		
Fasted male rabbit					
500	0	0	3	-	-
1000	0	3	3	1 – 3 day	-
LD_{50}: > 1000 mg/kg bw					
Fasted female rabbit					
500	0	0	3	-	-
1000	0	3	3	6 day	-
LD_{50}: > 1000 mg/kg bw					

Conclusion
This acute oral toxicity study of tebuconazole was investigated in accordance with GLP and OECD/EU guidelines. Tebuconazole was slightly toxic to fasted male and female rabbits after acute oral administration.

The following LD_{50} value was established.
LD_{50}: > 1000 mg/kg bw (male and female).

Under the conditions of the study with rabbits, no classification for acute oral toxicity is required. However, as higher doses than 1000 mg/kg bw were not tested, these results are not inconsistent with the current classification of tebuconazole as category 4 for acute oral toxicity (H302) according to Regulation (EC) No. 1272/2008.

B.6.2.1.4. *Summary of acute oral toxicity studies*

The acute oral toxicity of tebuconazole has been investigated in a total of seven studies in multiple species (3 in rats, 3 in mice and 1 in rabbits). The available studies, owned by the Bayer TF, were evaluated in the original DAR (2006), peer reviewed at EU level (EFSA Conclusion, 2008) and considered to be acceptable. Variable

results were obtained in these studies; however, 1 study in rats (LD_{50} = 1700 mg/kg bw in females) and 1 study in mice (LD_{50} = 1615 mg/kg bw in males) indicate that tebuconazole is of moderate acute oral toxicity and should be classified with category 4 (H302). This is consistent with the harmonised classification of tebuconazole.

B.6.2.2. Dermal

The acute dermal toxicity of tebuconazole has been investigated in two studies in rats. The available studies, owned by Bayer TF, were evaluated in the original DAR (2006), peer reviewed at EU level (EFSA Conclusion, 2008) and considered to be acceptable. These are re-summarised below. The results of these studies show that the acute dermal LD_{50} of tebuconazole is > 2000 mg/kg bw in rats. Classification for acute dermal toxicity is not required under the CLP Regulation (EC) No. 1272/2008.

A new acute dermal study has been provided for the purpose of renewal by the EU Tebuconazole Task Force. No new acute dermal studies have been provided by Bayer TF. The acute oral toxicity study submitted by EU Tebuconazole Task Force, which is a data matching study, confirms that tebuconazole is of low acute dermal toxicity, with a dermal LD_{50} in rats greater than 2060 mg/kg bw. Therefore, this study will be only briefly described in this RAR and it will not be relied upon.

B.6.2.2.1. *Acute dermal toxicity in rats*

a)

Previous evaluation:	In Tebuconazole DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.2.2/01
Study title	HWG 1608 Technical – Acute dermal toxicity study on rats
Test substance	(HWG 1608) Tebuconazole
Purity (%) **Batch no.**	98 (active substance) 816096181
Test animals	Male and female Sprague-Dawley rats (Crj: CD, SPF)
Groups	5 rats/sex/dose
Dose	2000 mg/kg bw
Route	Dermal (semi-occlusive conditions)
Vehicle	Polyethylene glycol 400
GLP	Yes
Guideline	OECD Guideline 402.
Deviation	The following deviations from the OECD-Guideline 402 (1987) occurred: - None
Acceptable	Acceptable
LD_{50}	>2000 mg/kg

Methods
Tebuconazole was administered dermal to groups of 5 male and 5 female Sprague-Dawley rats in a single dose at a level of 2000 mg/kg bw. The test substance was mixed with polyethylene glycol 400 and applied to the skin (semi occlusive conditions) for 24 hours. The post-treatment observation period lasted 14 days. Clinical signs were recorded several times on the day of treatment and further once a day. Body weight was recorded at the day of administration and further on a weekly basis. Autopsy was performed. A validated method of analysis for single exposure dermal studies is not required.

Results
No effects were observed with respect to mortality, bodyweight, clinical signs, skin irritation or pathological findings in rats dermally treated with tebuconazole in a single dose at a level of 2000 mg/kg bw.

Conclusion
The study was done according to the OECD-Guideline 402 (limit test). The acute dermal toxicity of Tebuconazole was tested in Sprague-Dawley rats at the dermal limit dose of 2000 mg/kg bw. No skin irritation findings were observed. The dermal toxicity of Tebuconazole is low.

An LD_{50} > 2000 mg/kg bw (male and female) was established based on no lethal effect at a maximal dose. Based on the results of this study, no classification for acute dermal toxicity is required (LD_{50} > 2000 mg/kg bw) according to Regulation (EC) No. 1272/2008.

b)

Previous evaluation:	In tebuconazole DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.2.2/02
Study title	HWG 1608 – Study for acute toxicity
Test substance	(HWG 1608) Tebuconazole
Purity (%) **Batch no.**	97.1 (active substance) 16001/83
Test animals	Male and female Wistar rats/Bor:WISW (SPF-Cpb)
Groups	5 rats/sex/dose
Dose	5000 mg/kg bw
Route	Dermal (occlusive dressing method)
Vehicle	Physiological saline solution
GLP	No – at the time the study was performed GLP was not compulsory.
Guideline	OECD Guideline 402.
Deviation	The following deviations from the OECD-Guideline 402 (1987) occurred: - None
Acceptable	Acceptable
LD_{50}	>5000 mg/kg

Methods
Tebuconazole was administered dermal to groups of 5 male and 5 female Wistar rats in a single dose at a level of 5000 mg/kg bw. The test substance was mixed with physiological saline solution and applied to the skin (occlusive dressing method) for 24 hours. The post-treatment observation period lasted 14 days. Clinical signs were recorded several times on the day of treatment and further once a day. Body weight was recorded at the day of administration and further on a weekly basis. Autopsy was performed. A validated method of analysis for single exposure dermal studies is not required.

Results
No effects were observed with respect to mortality, bodyweight, clinical signs, skin irritation or pathological findings in rats dermally treated with tebuconazole in a single dose at a level of 5000 mg/kg bw.

Conclusion
The study was done according to the OECD-Guideline 402. The acute dermal toxicity of tebuconazole was tested in Wistar rats at the dermal limit dose of 5000 mg/kg bw. No skin irritation findings were observed. The dermal toxicity of tebuconazole is low.

An LD_{50} > 5000 mg/kg bw (male and female) was established based on no lethal effect at a maximal dose. Based upon the results of this study, no classification for acute dermal toxicity is required (LD_{50} > 2000 mg/kg bw) according to Regulation (EC) No. 1272/2008.

c)

Previous evaluation:	None – submitted for the purpose of renewal (study owned by EU Tebuconazole Task Force)

Study ID	B.6.2.2/03
Study title	Acute dermal toxicity study of Tebuconazole TC in rats
Test substance	Tebuconazole TC
Purity (%)	97.1 - 97.2

Batch no.	20050122
Test animals	Male and female CD /CrI : CD(SD) rats
Groups	5 rats/sex/dose
Dose	2060 mg/kg bw
Route	Dermal (semi-occlusive)
Vehicle	Sesame oil
GLP	Yes
Guideline	OECD Guideline 402
Deviation	The following deviations from the OECD-Guideline 402 (1987) occurred: - None
Acceptable	Acceptable
LD_{50}	> 2060 mg/kg bw.

Methods
Tebuconazole TC was examined for acute toxicity after a single dermal application to rats. Tebuconazole was administered dermally to groups of 5 male and 5 female CD /CrI : CD(SD) rats in a single dose at a level of 2060 mg/kg bw. The test substance was mixed with sesame oil and applied to the skin (approximately $1/10^{th}$ body surface area, semi-occlusive dressing method) for 24 hours. The post-treatment observation period lasted 14 days. Clinical signs were recorded several times on the day of treatment and further once a day. Body weight was recorded at the day of administration and further on a weekly basis. Autopsy was performed. A validated method of analysis for single exposure dermal studies is not required.

Results
No effects were observed with respect to mortality, bodyweight, clinical signs, skin irritation or pathological findings in rats dermally treated with tebuconazole in a single dose at a level of 2060 mg/kg bw.

Table 6.2-6. Acute dermal toxicity

Dose [mg/kg bw]	Toxicological results			Duration of clinical signs	Time of death
	Dead	Toxic signs	Used		
Male rats					
2060	0	0	5	n/a	scheduled death
LD_{50}: >2060 mg/kg bw					
Female rats					
2060	0	0	5	n/a	scheduled death
LD_{50}: >2060 mg/kg bw					

Conclusion
The study was performed according to the OECD-Guideline 402. The acute dermal toxicity of tebuconazole was tested in rats at the dermal limit dose of 2060 mg/kg bw. No skin irritation findings were observed and no systemic toxicity was noted. The dermal toxicity of tebuconazole is low.

An LD_{50} > 2060 mg/kg bw (male and female) was established based on no lethal effect at a maximal dose. Based on the results of this study, no classification for acute dermal toxicity is required (LD_{50} > 2000 mg/kg bw) according to Regulation (EC) No. 1272/2008.

B.6.2.2.2. *Summary of acute dermal toxicity studies*

The acute dermal toxicity of tebuconazole has been investigated in two studies in rats. The available studies, owned by Bayer TF, were evaluated in the original DAR (2006), peer reviewed at EU level (EFSA Conclusion, 2008) and considered to be acceptable. A new acute dermal toxicity study in rats has been provided for the purpose of renewal by the EU Tebuconazole Task Force. These three studies in rats show that tebuconazole is of low acute dermal toxicity and no classification is required according to Regulation (EC) No. 1272/2008. This is consistent with the harmonised classification of tebuconazole.

B.6.2.3. Inhalation

The acute inhalation toxicity of tebuconazole has been investigated in two studies in rats. The available studies, owned by Bayer TF, were evaluated in the original DAR (2006), peer reviewed at EU level (EFSA Conclusion, 2008) and considered to be acceptable. These are re-summarised below. The results of these studies show that the acute inhalation 4hr-LC_{50} of tebuconazole is > 5.093 mg/kg bw in rats. Classification for acute inhalation toxicity is not required under Regulation (EC) No. 1272/2008.

A new acute inhalation study has been provided for the purpose of renewal by the Bayer TF, and an additional new study has been provided by the EU Tebuconazole Task Force. These new acute inhalation toxicity studies confirm that tebuconazole is of low acute inhalation toxicity, with an inhalation 4hr-LC_{50} in rats greater than 2.118 mg/L (max. attainable concentration Bayer TF), and greater than 5.0 mg/L (EU Tebuconazole Task Force).

B.6.2.3.1. *Acute inhalation toxicity in rats*

a)

Previous evaluation:	In tebuconazole DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.2.3.1/01
Study title	HWG 1608 – Study for acute inhalation to the rat
Test substance	(HWG 1608) Tebuconazole
Purity (%) **Batch no.**	96.2 Mixed batch Fl. no. 132
Test animals	Male and female Wistar rats/Bor:WISW (SPF-Cpb)
Groups	5 rats/sex/dose
Dose	Nominal concentration : 4000 mg/m³ (aerosol) Analytical concentration: 371 mg/m³ (aerosol), 5093 mg/m³ (dust)
Route	Inhalation (nose/head only)
Vehicle	Ethanol / polyethylene glycol E 400 (1:1) (aerosol) 20% HWG 1608 (w/v) in vehicle
GLP	Yes
Guideline	OECD Guideline 403.
Deviation	The following deviations from the OECD-Guideline 403 (2009) occurred: - The MMAD for dust tested = 12.8 µm + GSD (1.9 µm), 8% < 5 µm (Dust) which is above the recommended diameters 1 – 4 µm - body weights were not measured on day 1
Acceptable	Acceptable
LC_{50}	> 371 mg/m³ (aerosol = 0.371 mg/L) and > 5093 mg/m³ (dust = 5.093 mg/L)

Methods
The acute inhalation toxicity of tebuconazole was investigated in groups of 5 male and 5 female Wistar rats. The study was performed in inhalation chambers under dynamic conditions where rats were nose/head only exposed to the aerosol (371 mg/m³ which is the maximum technically producible concentration tolerated without clinical signs/mortality) and to the dust (5093 mg/m³ limit recommended in OECD 403). A control group was included (conditioned air with similar exposure conditions as were used for the test substance). Animals were exposed for 4 hours and a post-treatment observation period lasted for 14 days. Clinical signs (appearance and behaviour) were recorded on the day of treatment and in the observation period once a day. Body weight was recorded before exposure, on day 3, 7 and 14. Autopsy was performed at the end of the post-treatment period, and all animals were subjected to a gross pathology examination. Validation of the analytical methods used in this acute inhalation study, previously evaluated in the DAR (2006), is not required.

Results
Particle size
In the 4 hrs study the following characterization of the particles were observed:
MMAD = 1.4 µm ± GSD (1.4 µm), 100% ≤ 5 µm (Aerosol),
MMAD = 12.8 µm ± GSD (1.9 µm), 8% ≤ 5 µm (Dust)

Mortality
No mortality was observed during the study.

Body weight
Weight loss was observed in rats exposed to dust mainly on the third observation day.

Clinical signs
All the rats tolerated the treatment without clinical signs.

Pathology
The rats sacrificed at the end of the observation period did not provide any indications of grossly apparent lung or other organ damage.

Conclusion
This acute inhalation toxicity study of tebuconazole was performed in accordance with OECD-guideline 403. The study was performed at the maximum concentrations which could be obtained in the experimental design with respect to both aerosol and dust. Neither lethality nor clinical effects were observed. There were no indications of specific local lung toxicity or damage of organs at gross pathology. The study shows that tebuconazole has virtually no acute inhalation toxicity, either as aerosol or as dust.

A 4hr-LC_{50} > 371 mg/m³ = 0.371 mg/L (aerosol) and 4hr-LC_{50} > 5093 mg/m³ = 5.093 mg/L (dust) were established based on no lethal effect at the maximal achievable concentration.

Based on the results of this study in rats, no classification for acute inhalation toxicity is required (4hr-LC_{50} > 5.093 mg/L dust; highest achievable concentration) according to Regulation (EC) No. 1272/2008.

b)

Previous evaluation:	In tebuconazole DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.2.3/02
Study title	HWG 1608 - Study for acute toxicity (Report No. 12168) HWG 1608 - Study for acute inhalation toxicity to the rat to OECD guideline 403 (revised part of Report No. 12168)
Test substance	(HWG 1608) Tebuconazole
Purity (%) **Batch no.**	97.1 16001/83
Test animals	Male and female Wistar rats/Bor:WISW (SPF-Cpb)
Groups	5-10 rats/sex/dose
Dose	Nominal concentration: 100, 250, 2500, 5000 mg/m³ (aerosol exposure 1x4 hrs) 0, 100, 300, 1000 mg/m³ (aerosol exposure 5x6 hrs) Analytical concentration: 16, 49, 387, 818 mg/m³ (aerosol exposure 1x4 hrs) 0, 24, 60, 240 mg/m³ (aerosol exposure 5x6 hrs)
Route	Inhalation (nose/head only)
Vehicle	Ethanol / polyethylene glycol E 400 (1:1)
GLP	No – at the time the study was performed GLP was not compulsory.
Guideline	OECD Guideline 403.
Deviation	The following deviations from the OECD-Guideline 403 (2009) occurred: - MMAD > 4 µm - body weights not recorded on days 1 and 3 - More than 3 rats/sex/dose used in sighting/range finding study - top dose tested is lower than recommended limit (5 mg/L aerosols), expect 2 mg/L is achievable
Acceptable	Acceptable
LC_{50}	> 818 mg/m³ (1x4 hrs = 0.818 mg/L) and > 240 mg/m³ (5 times 6 hrs = 0.24 mg/L)

Methods
The acute inhalation toxicity of Tebuconazole was investigated in groups of 5-10 male and 5-10 female Wistar rats. The study was performed in inhalation chambers under dynamic conditions where rats were nose/head only

exposed. Animals were exposed for 1x4 hrs (acute inhalation) and 5x6 hrs (range-finding study). A vehicle control group was included in the 1x4 hrs study. The post-treatment observation period lasted for 14 days. Clinical signs (appearance and behaviour) were recorded on the day of treatment and in the observation period once a day. Body weight was recorded before exposure and on a weekly basis. Autopsy was performed at the end of the post-treatment period, and all animals were subjected to a gross pathology examination. Validation of the analytical methods used in this acute inhalation study, previously evaluated in the DAR (2006), is not required.

Results
Particle size
In the 1x4 hrs study particle size was approx. 50% ≤ 5 µm (not test-specific data).
In the 5x6 hrs study the following characterization of the particles were observed:
MMAD = 7.1 µm ± GSD (2.0µm), 31% ≤ 5 µm (100 mg/m³)
MMAD = 5.0 µm ± GSD (1.8 µm), 51% ≤ 5 µm (300 mg/m³)
MMAD = 4.6 µm ± GSD (1.8 µm), 55% ≤ 5 µm (1000 mg/m³)

Mortality
No mortality was observed during the study.

Body weight
There were no treatment related effects on body weight.

Clinical signs
In the 1x4 hrs study reduced motility (lassitude) was observed in the 250, 2500, 5000 mg/m³ dose groups.
In the 5x6 hrs study non-specific disturbed behaviour (lassitude) was observed in all groups.
Pathology
There were no indications of concentration-related grossly apparent lung or organ damage in the 1x4 hrs or in the 5x6 hrs study.

Conclusion
This acute inhalation toxicity study of tebuconazole aerosol was investigated in accordance with OECD-guideline 403. There were no indications of specific local lung toxicity or damage of organs at gross pathology. Tebuconazole exhibited a very slight toxicity to rats after acute inhalative administration to rats.

A 4hr-LC_{50} > 818 mg/m³ (1x4 hrs) and > 240 mg/m³ (5x6 hrs; range-finding study) for inhalation were established for male and female based on no lethal effect and no adverse effects, respectively, at the maximal achievable concentration.

Based on the results of this study in rats, no classification for acute inhalation toxicity is required (4hr-LC_{50} > 0.818 mg/L aerosol; maximum achievable concentration) according to Regulation (EC) No. 1272/2008.

c)

Previous evaluation:	None – submitted for the purpose of renewal (study owned by Bayer Task Force)

An additional acute inhalation toxicity study was conducted for a non-EU country.

Study ID	B.6.2.3/03
Study title	Tebuconazole (technical) - Acute inhalation toxicity in rats
Test substance	Tebuconazole
Purity (%) **Batch no.**	97.1 PF90146626
Test animals	Male and female wistar Hsd Cpb:WU rats
Groups	5 rats/sex/dose
Dose	2118 mg/m³
Route	Inhalation (nose/head only)
Vehicle	None
GLP	Yes

Guideline	OECD Guideline 403.
Deviation	The following deviations from the OECD-Guideline 403 (2009) occurred: - None
Acceptable	Acceptable
4hr-LC_{50}	> 2118 mg/m^3 (2.118 mg/L)

Methods
The acute inhalation toxicity of tebuconazole was investigated in groups of 5 male and 5 female Wistar (Hsd Cpb:WU) rats. The study was performed in inhalation chambers under dynamic conditions where rats were nose/head only exposed to tebuconazole aerosol (target concentration 5000 mg/m^3, actual 2118 mg/m^3 = maximum technically attainable concentration) for 4 hours. The post-treatment observation period lasted for 14 days. Clinical signs (appearance and behaviour) were recorded several times on the day of treatment, and once a day during the observation period. Body weight was recorded before exposure, on day 1, 3, 7 and weekly thereafter. Autopsy was performed at the end of the post-treatment period, and all animals were subjected to a gross pathology examination.

Results
Particle size
MMAD = 2.76 µm ± GSD (1.84 µm), 55% ≤ 3 µm (2118 mg/m^3, target concentration 5000 mg/m^3)

Mortality
No mortality was observed during the study.

Body weight
Temporary decrease in body weights in females in the exposure group compared to controls were present but not statistically significant.

Table 6.2-7. Body weights

Target concentration (mg/m^3)		Day 0	1	3	7	14
Males	0	185.2	183.2	201.4	230.8	272.8
	5000	192.0	187.8	202.2	225.6	260.8
Females	0	183.6	180.6	184.8	195.8	211.8
	5000	179.8	180.2	183.4	193.0	205.8

Clinical signs
In the control study, all rats tolerated the exposure without specific signs. After exposure observations included nonspecific effects including an ungroomed hair-coat on the post exposure days 1-2. From post exposure day 2 onwards all rats appeared to be indistinguishable from the controls. Statistical comparisons between the control and the exposure group revealed some minor although significant changes in body temperature. However, the extent of change was too small for mild hypothermia to be of any toxicological significance.

Pathology
Macroscopic findings were essentially indistinguishable between exposure and control groups

Table 6.2-8. Inhalation toxicity

Target concentration (mg/m^3)	Toxicological results: Dead	Toxic signs	Used	Duration of clinical signs	Time of death	Rectal Temperature (°C)
Males						
0	0	0	5		--	38.0
5000	0	3	5	1d – 2d	--	36.2**
LC_{50}: 2.118 mg/m^3						
Females						
0	0	0	5		--	38.0
5000	0	3	5	1d – 2d	--	37.1**

LC_{50}: 2118 mg/m³

* = p < 0.05, ** = p < 0.01

Conclusion
Tebuconazole proved to have no acute inhalation toxicity in rats. The signs observed were non-specific and transient. A 4hr-LC_{50} > 2.118 mg/L (2118 mg/m³) was established for male and female based on no lethal effect and no adverse effects, respectively, at the maximal achievable concentration.

Based on the results of this study in rats, no classification for acute inhalation toxicity is required (4hr-LC_{50} > 2.118 mg/L aerosol; highest achievable concentration) according to Regulation (EC) No. 1272/2008.

d)

Previous evaluation:	None – submitted for the purpose of renewal (study owned by EU Tebuconazole Task Force)

An additional inhalation study was provided by the EU Tebuconazole Task Force.

Study ID	B.6.2.3/04
Study title	Acute inhalation study of Tebuconazole TC in rats
Test substance	Tebuconazole
Purity (%) **Batch no.**	97.1-97.2 20050122
Test animals	Male and female CD /CrI : CD(SD) rats
Groups	5 rats/sex/dose
Dose	5.00 mg/L air (limit test)
Route	Inhalation (nose/head only)
Vehicle	Sesame oil
GLP	Yes
Guideline	OECD Guideline 403.
Deviation	The following deviations from the OECD-Guideline 403 (2009) occurred: - body weights were not recorded and days 1 and 3 - MMAD> 1-4 mikrometer
Acceptable	Acceptable
LC_{50}	> 5.0 mg/L aerosol, the maximal achievable concentration

Methods
The acute inhalation toxicity of tebuconazole was investigated in groups of 5 male and 5 female (CD /CrI : CD(SD) rats. The study was performed in inhalation chambers under dynamic conditions where rats were nose/head only exposed to tebuconazole aerosol (5.0 mg/L limit as described in OECD 403) for 4 hours. The post-treatment observation period lasted for 14 days. Clinical signs (appearance and behaviour) were recorded several times on the day of treatment, and at least once a day during the observation period. Body weight was recorded before exposure and weekly thereafter. Autopsy was performed at the end of the post-treatment period, and all animals were subjected to a gross pathology examination.

Results
Particle size
MMAD = 16.324 µm + GSD (2.38 µm (5000 mg/m³)

Mortality
No mortality was observed during the study.

Body weight
Body weight gain was considered to be normal.

Clinical signs
No clinical signs of toxicity were observed throughout the duration of the study.

Pathology
No pathological findings were noted at necroscopy.

Table 6.2-9. Inhalation toxicity

Concentration (mg/m^3)	Toxicological results			Duration of clinical signs	Time of death
	Dead	Toxic signs	Used		
Male					
5000	0	0	5	n/a	Scheduled sacrifice
LC_{50}: > 5.0 mg/L					
Female					
5000	0	0	5	n/a	Schedule sacrifice
LC_{50}: > 5.0 mg/L					

Conclusion
Tebuconazole proved to have no acute inhalation toxicity in rats. A 4hr-LC_{50} > 5.0 mg/L aerosol (5000 mg/m^3) was established for male and female based on no lethal effect and no adverse effects, respectively, at the maximal achievable concentration.

Based on the results of this study in rats, no classification for acute inhalation toxicity is required (4hr-LC_{50} > 5.0 mg/L aerosol, the maximal achievable concentration) according to Regulation (EC) No. 1272/2008.

B.6.2.3.2. *Summary of acute inhalation toxicity studies*

The acute inhalation toxicity of tebuconazole has been investigated in two studies in rats. The available studies, owned by Bayer TF, were evaluated in the original DAR (2006), peer reviewed at EU level (EFSA Conclusion, 2008) and considered to be acceptable. A new acute inhalation study has been provided for the purpose of renewal by the Bayer TF, and an additional new study has been provided by the EU Tebuconazole Task Force. Overall, these 4 studies in rats show that tebuconazole is of low acute inhalation toxicity (4hr-LC_{50} > 5000 mg/l aerosol) and that classification according to the CLP Regulation is not required. This is consistent with the harmonised classification of tebuconazole.

B.6.2.4. Skin irritation

The potential for tebuconazole to induce skin irritation has been investigated in two studies in rabbits. The available studies, owned by the Bayer TF, were evaluated in the original DAR (2006), peer reviewed at EU level (EFSA Conclusion, 2008), and considered acceptable. These are re-summarised below. A new skin irritation study has been provided for the purpose of renewal by the EU Tebuconazole Task Force. This new study confirms that tebuconazole is not a skin irritant. This new study, which is a data-matching study, represents duplicate vertebrate testing and will not be relied upon.

B.6.2.4.1. *Acute skin irritation in rabbits*

a)

Previous evaluation:	In tebuconazole DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.2.4/01
Study title	HWG 1608 - Study for acute toxicity HWG 1608 – c. n.: Tebuconazole (proposed) – Study for skin and eye irritation/corrosion in rabbits (Addendum to Report No. 12168)
Test substance	(HWG 1608-technical grade) Tebuconazole
Purity (%) **Batch no.**	97.1 16001/83
Test animals	Male and female New Zealand White rabbit, HC:NZW
Groups	3 rabbits/sex/dose
Dose	500 mg

Route	Dermal (semi-occlusive)
Vehicle	Test substance was moistened with water.
GLP	No – GLP was not compulsory at the time the study was performed.
Guideline	OECD Guideline 404.
Deviation	The following deviations from the OECD-Guideline 404 (2015) occurred: - Conducted as one test using three animals as opposed to an initial and confirmatory test
Acceptable	Acceptable
Result	Non irritating

Methods
The acute skin irritation test (Patch-Test) of tebuconazole was investigated in 3 adult rabbits. The treated skin area was approx. 6 cm^2. The test substance (0.5g) was applied to the skin by a patch and held in contact with the skin by means of a semi-occlusive dressing. The untreated skin served as a control. Animals were exposed for 4 hours. The skin sites were evaluated before and 60 minutes, 24, 48, 72 hours and 7 days after patch removal. The evaluation of the skin irritation was based on the approved grading system (Draize) of the level of reddening (erythema/eschar formation) and swelling (oedema formation). A validated method of analysis for single exposure skin and eye irritation studies is not required.

Results
Mortality
No mortality was observed during the study.

Body weight
There were no treatment related effects on body weight.

Clinical signs
No skin irritation was observed (average erythema and oedema formation was 0,0).

Table 6.2-10. Skin irritation

Time after patch removal	Incidence of irritation		Severity irritation (mean score)
	Erythema	Oedema	
1 hour	0/3	0/3	0
24 hours	0/3	0/3	0
48 hours	0/3	0/3	0
72 hours	0/3	0/3	0
7 days	0/3	0/3	0

Conclusion
This acute skin irritation study of tebuconazole was investigated in accordance with OECD-guideline 404. There were no irritant effects (erythema and oedema formation) of tebuconazole.

The results of this study in rabbits indicate that the test substance was not a primary skin irritant. No classification for skin irritation is required according to Regulation (EC) No. 1272/2008.

b)

Previous evaluation:	In tebuconazole DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.2.4/02
Study title	Primary dermal irritation of technical grade Folicur in rabbits
Test substance	(HWG 1608-technical grade) Tebuconazole
Purity (%) **Batch no.**	96.6 (active substance) 86R0082I
Test animals	Male and female New Zealand White rabbit,
Groups	3 rabbits/sex/dose
Dose	500 mg

Route	Dermal
Vehicle	Test substance was moistened with water.
GLP	Yes
Guideline	OECD Guideline 404.
Deviation	The following deviations from the OECD-Guideline 404 (2015) occurred: - Conducted as one test using three animals as opposed to an initial and confirmatory test
Acceptable	Acceptable
Result	Non irritating

Methods
The acute skin irritation test (Patch-Test) of Tebuconazole was investigated in 6 adult rabbits. The treated skin area was approx. 6 cm^2. The test substance (0.5g) was applied to the skin by a patch and held in contact with the skin under occlusive patch conditions. The untreated skin served as a control. Animals were exposed for 4 hours. The skin sites were evaluated before and 60 minutes, 24, 48, 72 hours after patch removal. The evaluation of the skin irritation was based on the approved grading system (Draize) of the level of reddening (erythema/eschar formation) and swelling (oedema formation). A validated method of analysis for single exposure dermal irritation studies is not required.

Results
There was no mortality during the study, and no skin irritation was observed (primary irritation index was 0.0).

Table 6.2-11. Skin irritation

Time after patch removal	Incidence of irritation		Severity irritation (mean score)
	Erythema	Oedema	
1 hour	0/3	0/3	0
24 hours	0/3	0/3	0
48 hours	0/3	0/3	0
72 hours	0/3	0/3	0

Conclusion
This acute skin irritation study of tebuconazole was investigated in accordance with OECD guideline 404. There were no irritant effects (erythema and oedema formation) of tebuconazole.

The results of this study in rabbits indicate that tebuconazole was not a primary skin irritant. No classification for skin irritation is required according to Regulation (EC) No. 1272/2008.

c)

Previous evaluation:	None – Submitted for the purpose of renewal (EU Tebuconazole Task Force)

Study ID	B.6.2.4/03
Study title	Acute dermal irritation/corrosion test (patch test) of Tebuconazole TC in rabbits
Test substance	Tebuconazole,
Purity (%) **Batch no.**	97.1 - 97.2 20050122
Test animals	3 male Himalayan rabbits
Groups	3/dose
Dose	500 mg
Route	Dermal (semi-occlusive conditions)
Vehicle	Aqua ad iniectabilia
GLP	Yes
Guideline	OECD Guideline 404.
Deviation	The following deviations from the OECD-Guideline 404 (2015) occurred: - None
Acceptable	Acceptable
Result	Non irritating

Methods
The acute skin irritation potential of tebuconazole was investigated in 3 male adult rabbits. An initial test was conducted with one animal, then a confirmatory test was conducted with an additional two animals. The test substance (0.5g) was applied to the skin (approx. 6 cm^2) and held in contact with the skin under semi-occlusive patch conditions. The untreated skin served as a control. Animals were exposed for 4 hours. The skin sites were evaluated before and 60 minutes, 24, 48, 72 hours after patch removal. The evaluation of the skin irritation was based on the approved grading system (Draize) of the level of reddening (erythema formation) and swelling (oedema formation). A validated method of analysis for single exposure dermal irritation studies is not required.

Results
Mortality
No mortality was observed during the study.

Body weight
There were no treatment related effects on body weight.

Clinical signs
No skin irritation was observed (average erythema and oedema formation was 0,0)

Table 6.2-12. Skin irritation

Time after patch removal	Incidence of irritation		Severity irritation (mean score)
	Erythema	Oedema	
30 – 60 mins	0/3	0/3	0
24 hours	0/3	0/3	0
48 hours	0/3	0/3	0
72 hours	0/3	0/3	0

Conclusion
This acute skin irritation study of tebuconazole was performed in accordance with OECD guideline 404. There were no irritant effects (erythema and oedema formation) of tebuconazole.

The results of this study in rabbits indicate that tebuconazole was not a primary skin irritant. No classification for skin irritation is required according to Regulation (EC) No. 1272/2008.

B.6.2.4.2. *Summary of skin irritation studies*

The potential for tebuconazole to induce skin irritation has been investigated in two studies in rabbits. The available studies, owned by the Bayer TF, were evaluated in the original DAR (2006), peer reviewed at EU level (EFSA Conclusion, 2008), and considered acceptable. A new skin irritation study has been provided for the purpose of renewal by the EU Tebuconazole Task Force. Overall, these three studies show that tebuconazole is not a skin irritant and hence classification according to Regulation (EC) No. 1272/2008 is not required. This is consistent with the harmonised classification of tebuconazole.

B.6.2.5. Eye irritation

The potential for tebuconazole to cause eye irritation has been investigated in two studies in rabbits . The available studies, owned by the Bayer TF, were evaluated in the original DAR (2006), peer reviewed at EU level (EFSA Conclusion, 2008), and considered acceptable. These are re-summarised below. A new eye irritation study has been provided for the purpose of renewal by the EU Tebuconazole Task Force. This new study confirms that tebuconazole is only slightly irritating to the eye (but requiring no classification). This new study, which is a data-matching study, represents duplicate vertebrate testing and will not be relied upon.

B.6.2.5.1. *Acute eye irritation in rabbits*

a)

Previous evaluation:	In tebuconazole DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.2.5.1/01
Study title	Primary Eye Irritation of FOLICUR® (HWG 1608) Technical in Albino Rabbits,
Test substance	(Folicur-technical grade) Tebuconazole
Purity (%) **Batch no.**	96.3 (active substance) 86R0082I
Test animals	Male and Female New Zealand White rabbit
Groups	3 rabbits/sex/dose
Dose	0.1 g
Route	Into the conjunctival sac of the left eye
Vehicle	None, undiluted application
GLP	Yes
Guideline	OECD Guideline 405.
Deviation	The following deviations from the OECD-Guideline 405 (2012) occurred: - unclear whether topical anaesthetics and systemic analgesics were used - Conducted as one test using three animals as opposed to an initial and confirmatory test
Acceptable	Acceptable
Result	Not irritating to eyes

Methods
The acute eye irritation test of tebuconazole was investigated in 3 male and 3 female rabbits. A single dose of 0.1 g was applied by instillation into the conjunctival sac of the left eye of the rabbits. The right eye, which remained untreated, served as a control. The test period was 72 h and no rinse was performed after application of test substance. The eyes were examined ophthalmoscopically before the administration and 1, 24, 48, 72, hours after the administration. Additional examinations occurred on days 7, 8, 14, and 21, in order to characterize the time-course and reversibility of lesions. The eye reactions were observed and registered and the effects were graded based on the approved grading system of the level of ocular irritation scores (Draize). The condition of the cornea (opacity and area affected), iris (hyperaemia, reaction to light) and conjunctivae (erythema and chemosis) were evaluated. A validated method of analysis for single exposure eye irritation studies is not required.

Results
No mortality occurred during the study. No systemic intolerance reactions were observed.

Eye irritation
There were no signs of corneal opacities or lesions involving the iris in any animal during the study. All six rabbits developed redness (grade 1), chemosis (grade 1-2) and discharge (grade 2-3) of the conjunctiva 1-24 h after dosing. Chemosis and discharge had resolved in five animals by 72 h after dosing and in all animals by day 7. Redness had resolved in five rabbits by 72 hours after dosing and in the one remaining animal by day 8.

The average scores for all six rabbits, which is based on individual scoring values were: Cornea (24h-72h: 0.00), Iris (24h-72h: 0.00), Redness (24h-72h: 0.78), Chemosis (24h-72h: 0.50), discharge (24h-72h: 0.55)

Table 6.2-13. Results table - Males

Males Animal	Effects	1 h	24 h	48 h	72 h	Mean scores (24, 48 and 72 h)	Reversible day
1	Corneal opacity	0	0	0	0	0	-
	Iritis	0	0	0	0	0	-
	Redness conjunctivae	1	1	1	0	0.67	3
	Chemosis conjunctivae	0	1	0	0	0.33	2
	Discharge	2	0	0	0	0	1
2	Corneal opacity	0	0	0	0	0	-
	Iritis	0	0	0	0	0	-
	Redness conjunctivae	1	1	1	0	0.67	3
	Chemosis conjunctivae	1	1	0	0	0.33	2
	Discharge	3	0	0	0	0	1

3	Corneal opacity	0	0	0	0	0	-
	Iritis	0	0	0	0	0	-
	Redness conjunctivae	1	1	1	0	0.67	3
	Chemosis conjunctivae	1	1	0	0	0.33	2
	Discharge	3	3	0	0	1	2

Table 6.2-14. Results table - Females

Females Animal	**Effects**	**1 h**	**24 h**	**48 h**	**72 h**	**Mean scores (24, 48 and 72 h)**	**Reversible day**
1	Corneal opacity	0	0	0	0	0	n/a
	Iritis	0	0	0	0	0	n/a
	Redness conjunctivae	1	1	1	1	1	8
	Chemosis conjunctivae	2	2	1	1	1.33	4-7
	Discharge	3	3	1	0	1.33	3
2	Corneal opacity	0	0	0	0	0	n/a
	Iritis	0	0	0	0	0	n/a
	Redness conjunctivae	1	1	1	0	0.67	3
	Chemosis conjunctivae	2	1	0	0	0.33	2
	Discharge	3	3	0	0	1	2
3	Corneal opacity	0	0	0	0	0	n/a
	Iritis	0	0	0	0	0	n/a
	Redness conjunctivae	1	1	1	1	1	4-7
	Chemosis conjunctivae	1	1	0	0	0.33	2
	Discharge	2	0	0	0	0	1

n/a = not applicable

Conclusion
Tebuconazole was tested in six New Zealand White rabbits for its potential to cause eye irritation. Tebuconazole caused discharge, redness and swelling of the conjunctiva that resolved by eight days after dosing. Tebuconazole did not cause corneal opacities or lesions of the iris.

Based on these results in rabbits, tebuconazole is slightly irritating to the eyes; however, no classification for eye irritation is required according to Regulation (EC) No. 1272/2008.

b)

Previous evaluation:	In tebuconazole DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.2.5.1/02
Study title	HWG 1608 – Study for acute toxicity (Report No. 12168) HWG 1608 – c. n.: Tebuconazole (proposed) – Study for skin and eye irritation/corrosion in rabbits (Addendum to Report No. 12168)
Test substance	(HWG 1608-technical grade) Tebuconazole
Purity (%) **Batch no.**	97.1 16001/83
Test animals	Male New Zealand White rabbit, HC:NZW
Groups	3/dose
Dose	100 µl (weight 50 mg)
Route	Into the conjunctival sac of the left eye

Vehicle	None, undiluted application
GLP	No – GLP was not compulsory at the time the study was performed.
Guideline	OECD Guideline 405.
Deviation	The following deviations from the OECD-Guideline 405 (2012) occurred: -
Acceptable	Acceptable
Result	Not irritating to eyes

Methods

The acute eye irritation test of Tebuconazole was investigated in 3 male rabbits. A single dose of 50 mg (100 µl) was applied by instillation into the conjunctival sac of the eye (the other eye, which remained untreated, served as a control). The test period was 24 hours. The eyes were examined ophthalmoscopically before the administration and 1, 24, 48, 72, hours and 7 days after the administration. The eye reactions were observed and registered and the effects were graded based on the approved grading system of the level of ocular irritation scores (Draize). The condition of the cornea (opacity and area affected), iris (hyperaemia, reaction to light) and conjunctivae (erythema and chemosis) were evaluated. The dacrryorrhoea (tear flow) was also assessed. A validated method of analysis for single exposure eye irritation studies is not required.

Results

Mortality

No mortality was observed during the study.

Eye irritation

There were no signs of corneal opacities or lesions involving the iris in any animal during the study. Reddening of conjunctiva was observed in one animal (average score 0.33; reversible at 48 hours). No chemosis or discharge was observed.

Table 6.2-15. Results table

Males Animal	Effects	1 h	24 h	48 h	72 h	Mean scores (24, 48 and 72 h)	Reversible day
1	Corneal opacity	0	0	0	0	0	n/a
	Iritis	0	0	0	0	0	n/a
	Redness conjunctivae	2	1	0	0	0.33	2
	Chemosis conjunctivae	1	0	0	0	0	1
	Discharge	1	0	0	0	0	1
2	Corneal opacity	0	0	0	0	0	n/a
	Iritis	0	0	0	0	0	n/a
	Redness conjunctivae	2	0	0	0	0	1
	Chemosis conjunctivae	1	0	0	0	0	1
	Discharge	0	0	0	0	0	n/a
3	Corneal opacity	0	0	0	0	0	n/a
	Iritis	0	0	0	0	0	n/a
	Redness conjunctivae	2	0	0	0	0	1
	Chemosis conjunctivae	1	0	0	0	0	1
	Discharge	0	0	0	0	0	n/a

n/a = not applicable

Clinical signs

No systemic intolerance reactions were observed during and after the administration.

Conclusion

Tebuconazole was tested in three New Zealand White rabbits for its potential to cause eye irritation. The test material did not cause corneal opacities or lesions of the iris.

Based on these results of this study in rabbits, tebuconazole is not irritating to eyes. No classification for eye irritation is required according to Regulation (EC) No. 1272/2008.

c)

Previous evaluation:	None – submitted for the purpose of renewal (study owned by EU Tebuconazole Task Force)

Study ID	B.6.2.5.1/03
Study title	Acute eye irritation/corrosion test of Tebuconazole TC
Test substance	Tebuconazole
Purity (%) **Batch no.**	97.1 - 97.2 20050122
Test animals	Male Himalayan rabbits
Groups	3/dose
Dose	100 mg
Route	Into the conjunctival sac of the left eye
Vehicle	None
GLP	Yes
Guideline	OECD Guideline 405.
Deviation	The following deviations from the OECD-Guideline 405 (2012) occurred: -
Acceptable	Acceptable
Result	Not irritating to eyes

Methods
The acute eye irritation potential of tebuconazole was investigated in 3 male rabbits. A single dose of 100 mg was applied by instillation into the conjunctival sac of the right eye (the other eye, which remained untreated, served as a control). The eyes were examined ophthalmoscopically before the administration and 1, 24, 48, 72, hours and 4 days after the administration. The eye reactions were observed and registered and the effects were graded based on the approved grading system of the level of ocular irritation scores (Draize). The condition of the cornea (opacity and area affected), iris (hyperaemia, reaction to light) and conjunctivae (erythema and chemosis) were evaluated. A validated method of analysis for single exposure eye irritation studies is not required.

Results
Mortality
No mortality was observed during the study.

Eye irritation
Corneal opacity (grade 1) was observed in one animal 24 to 72 hours after instillation: the fluorescein test performed 24 hours after instillation revealed corneal staining in this animal. Conjunctival redness (grade 1) was observed in two animals (24 and 48 hours in animal no. 3, and 24 until 72 hours in animal no. 1) after instillation. Chemosis (grade 1) was observed in one animal 24 hours after instillation. The iris was not affected.

Table 6.2-16. Eye irritation

Males Animal	Effects	1 h	24 h	48 h	72 h	Mean scores (24, 48 and 72 h)	Reversible day
1	Corneal opacity	0	1	1	1	1	4
	Iritis	0	0	0	0	0	n/a
	Redness conjunctivae	0	1	1	1	1	4
	Chemosis conjunctivae	0	0	0	0	0	n/a
2	Corneal opacity	0	0	0	0	0	n/a
	Iritis	0	0	0	0	0	n/a
	Redness conjunctivae	0	0	0	0	0	n/a
	Chemosis conjunctivae	0	0	0	0	0	n/a

3	Corneal opacity	0	0	0	0	0	n/a
	Iritis	0	0	0	0	0	n/a
	Redness conjunctivae	0	1	1	0	0.67	2
	Chemosis conjunctivae	0	1	0	0	0.33	1

*scores in the range of 0 to 4 for cornea opacity and chemosis, 0 to 3 for redness of conjunctivae and 0 to 2 for iritis
n/a = not applicable

Clinical signs
No systemic intolerance reactions were noted during and after the administration.

Conclusion
Tebuconazole was tested in three New Zealand White rabbits for its potential to cause eye irritation. Tebuconazole caused corneal opacities, redness and swelling of the conjunctiva that resolved by four days after dosing. The test material did not cause lesions of the iris.

Based on these results of this study in rabbits, tebuconazole is only slightly irritating to eyes. However, no classification for eye irritation is required according to Regulation (EC) No. 1272/2008.

B.6.2.5.2. ***Summary of eye irritation studies***

The potential for tebuconazole to cause eye irritation has been investigated in two studies in rabbits. The available studies, owned by the Bayer TF, were evaluated in the original DAR (2006), peer reviewed at EU level (EFSA Conclusion, 2008), and considered acceptable. A new eye irritation study has been provided for the purpose of renewal by the EU Tebuconazole Task Force. Overall, these three studies in rabbits show that tebuconazole is only slightly irritating to eye but that no classification is required according to Regulation (EC) No. 1272/2008. This is consistent with the harmonised classification of tebuconazole.

B.6.2.6. Skin sensitization

Four studies investigating the skin sensitisation potential of tebuconazole have been conducted: two guinea pig maximisation tests, and two Buehler Patch tests. The available studies, owned by the Bayer TF, were evaluated in the original DAR (2006), peer reviewed at EU level (EFSA Conclusion, 2008), and considered acceptable. These are re-summarised below. A new skin sensitisation study (guinea pig maximisation test) has been provided for the purpose of renewal by the EU Tebuconazole Task Force. This new study confirms that tebuconazole is not a skin sensitiser. This new study, which is a data-matching study, represents duplicate vertebrate testing and will not be relied upon.

B.6.2.6.1. ***Skin sensitisation in guinea pigs (Guinea Pig Maximization Test)***

a)

Previous evaluation:	In tebuconazole DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.2.6.1/01
Study title	HWG 1608 – Study for the skin sensitization effect in guinea pigs (Guinea Pig Maximization Test according to Magnusson and Kligman)
Test substance	(HWG 1608) Tebuconazole
Purity (%) **Batch no.**	96.9 (active substance) 278479023
Test animals	Female Guinea pigs/Hsd Poc:DH (SPF-bred)
Groups	35 guinea pigs (20 in test group, 10 in control group, 5 in range finding)
Dose	Intradermal induction (0.1 mL/animal, 5 % suspension) Topical induction (0.5 mL/patch, 50%) Topical challenge (0.5 mL/patch, 40%)
Route	Intradermal, dermal
Vehicle	Physiological saline solution

GLP	Yes
Guideline	OECD Guideline 406.
Deviation	The following deviations from the OECD-Guideline 406 (1992) occurred: - sodium lauryl sulphate was not applied before topical induction (required as other acute studies have shown that tebuconazole is not a skin irritant). This was applied in another study at lower dose (25% TBZ, Heimann (1983)) and some post challenge reactions were seen.
Acceptable	Acceptable
Result	Not sensitising

Methods
The skin sensitisation effect of tebuconazole was investigated in female guinea pigs (maximization test of Magnusson and Kligman). A dose-range finding study (5 animals) was performed to estimate doses for the induction (highest dose which causes mild irritation was identified as 50% - this induced skin irritation in ¼ guinea pigs tested) and first challenge (highest non-irritating dose was identified as the next lower dose tested: 40%). Based on these results two groups of animals were included, one test group (20 animals) and one control group (10 animals). Intradermal injections were given to the test group (0.1 mL/animal, 5% test substance with adjuvant) and the control group (0.1 mL/animal without test substance and with adjuvant). The topical induction was performed one week after the intradermal induction with 0.5 mL 50 % test substance (test group) and without test substance (control group) in a 48 hour exposure period. The challenge was performed three weeks respectively after the intradermal induction with 0.5 mL 40% test substance to the test and control group in a 24 hours exposure period. Observations on skin effects, clinical signs and body weight were performed. The skin reactions were assessed after 48 and 72 hours. A validated method of analysis for skin sensitisation studies is not required.

Results
No mortality was observed during the study. There were no treatment related changes in body weight. No clinical signs were observed.

Skin sensitisation
No skin effects in the test substance group and in the control group.

The methodological reliability of the test, and sensitivity of the strain used, was confirmed using 2-mercaptobenzothiazole formulated with physiological NaCl, containing 2 % v/v Cremophor EL (2.5 % intradermal induction, 40 % topical induction, 40 % challenge). After challenge 60 % of 2-mercaptobenzothiazole (positive control) test animals exhibited dermal reactions and the sensitivity and reliability of the technique was confirmed.

Conclusion
This skin sensitisation potential of tebuconazole was investigated in accordance with GLP and OECD/EU guidelines.

Under the conditions of this Guinea Pig Maximization Test according to Magnusson and Kligman (GPMT), tebuconazole showed no skin-sensitising potential. No classification for skin sensitisation is required according to Regulation (EC) No. 1272/2008.

b)

Previous evaluation:	In tebuconazole DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.2.6.1/02
Study title	HWG 1608 – Study for skin-sensitising effect on guinea pigs (Report No. 12024)
Test substance	(HWG 1608-technical grade) Tebuconazole
Purity (%) **Batch no.**	97.1 16001/83
Test animals	Male Guinea pigs/Pirbright white W58
Groups	40 guinea pigs (20 in test group, 20 in control group)
Dose	Intradermal induction (0.1 mL/animal, 1% suspension)

	Topical induction (25%) Topical challenge (25%)
Route	Intradermal, dermal
Vehicle	Distilled water containing 2 % Cremophor EL glycol
GLP	No – GLP was not compulsory at the time the study was performed.
Guideline	OECD Guideline 406.
Deviation	The following deviations from the OECD-Guideline 406 (1992) occurred: - skin reactions assessed at 24 and 48 h not 48 and 72h - Formulated in water not saline - Highest dose tested in pilot study (25%) showed no irritation. Questions over whether appropriate dose tested - Assessment scores not done to Magnusson and Kligman in guideline - No positive control results presented
Acceptable	Acceptable
Result	Not sensitising

Methods
The skin sensitisation effect of tebuconazole was investigated in guinea pigs (maximization test of Magnusson and Kligman). A dose-range finding study (4 animals) was performed to estimate doses for the induction (highest dose which causes mild irritation; note that highest dose tested didn't cause irritation) and first challenge (highest non-irritating dose). Based on these results two groups of animals were included, one test group (20 animals) and one control groups (20 animals). Intradermal injections were given the test group (0.1 mL/animal, 1% test substance with adjuvant) and the control group (0.1 mL/animal without test substance and with adjuvant). The topical induction was performed one week after the intradermal induction with 25% test substance (test group) (not irritating to the skin and the animals were therefore prepared with 10% sodium laurylsulphate in vaseline) and without test substance (control group) in a 48 hour exposure period. The challenge was performed three weeks respectively after the intradermal induction with 25% test substance applied via a patch to the left flank of the test and control group in a 24 hours exposure period. A control patch was applied to the right flank of test and control animals for comparison. Observations on skin effects, clinical signs and body weight were performed. The skin reactions were assessed after 24 and 48 hours. Sensitisation was estimated by subtracting the number of animals reacting with irritation on the control right side, from the number reacting with irritation on the test substance left side. A validated method of analysis for skin sensitisation studies is not required.

Results
5 animals in the control group and 2 animals in the treatment group died during the study. No treatment related changes in body weight or any other clinical signs were observed.

Skin sensitisation
Evaluation revealed the same number of positive skin reactions in the test substance group on compound (left) and control (right) flanks (positively reacting animals in test compound group: 8 compound and 8 control). Evaluation revealed the same number of positive skin reactions in the control group on compound (left) and control (right) flanks (positively reacting animals in control group: 3 compound and 3 control).

Table 6.2-17. Skin sensitisation study of tebuconazole TC

	Tebuconazole TC group		**Total number of animals affected**	**Adjusted value (test flank – control flank)**
	24 hours	**48 hours**		
After challenge (test substance dressing)	8/18 (2 died)	2/18 (2 died)	8	0
After challenge (control dressing)	7/18 (2 died)	2/18 (2 died)	8	
	Test Vehicle Control Group			

	Tebuconazole TC group		Total number of animals affected	Adjusted value (test flank – control flank)
After challenge (test substance dressing)	3/16 (4 died[+])	1/16 (4 died[+])	3	0
After challenge (control dressing)	3/16 (4 died[+])	1/16 (4 died[+])	3	

* Number of animals with positive dermal response (scores of 1-3) /number of animals in dose group.
[+] An additional animal died following evaluation but before recording of final weights, Total 5 died in control group.

Conclusion
This skin sensitising study of tebuconazole was performed in accordance with OECD/EU guidelines.

Under the conditions of this Guinea Pig Maximization Test according to Magnusson and Kligman (GPMT), tebuconazole showed no skin-sensitising potential. No classification for skin sensitisation is required according to Regulation (EC) No. 1272/2008.

c)

Previous evaluation:	None – submitted for the purpose of renewal (study owned by EU Tebuconazole Task Force)

Study ID	B.6.2.6.1/03
Study title	Skin sensitisation test of Tebuconazole TC in guinea pigs according to Magnusson and Kligman (Maximisation test)
Test substance	Tebuconazole
Purity (%) **Batch no.**	97.1 - 97.2 20050122
Test animals	Male and female Dunkin Hartley Guinea pigs
Groups	8 males preliminary study, 10 female test group, 5 females control group.
Dose	Intradermal induction (0.1 mL/animal, 10% suspension) Topical induction (50%) Topical challenge (10%)
Route	Intradermal, dermal
Vehicle	Sesame oil
GLP	Yes
Guideline	OECD Guideline 406.
Deviation	The following deviations from the OECD-Guideline 406 (1992) occurred: - 20 test and 10 control animals strongly recommended. 10 test and 5 control used though this is in line with the minimum
Acceptable	Acceptable
Result	Not sensitising

Methods
The potential of Tebuconazole technical concentrate to provoke skin sensitisation reactions was investigated in Guinea pigs according to the Magnusson and Kligman Maximisation maximization test. A dose-range finding study (8 animals: 2 intradermal 0.01 - 10 % tested, and 6 topical 0.5 – 50 % tested) was performed to estimate doses for the induction (highest dose which causes mild irritation – 50 %) and first challenge (highest non-irritating dose – 10 %). Intradermal injections were given the test group (0.1 mL/animal, 10 % test substance) and the control group (0.1 mL/animal without test substance). The topical induction was performed one week after the intradermal induction with 50 % test substance (test group) (not irritating to the skin and the animals were therefore prepared with 10 % sodium laurylsulphate in vaseline) and without test substance (control group) in a 48 hour exposure period. The challenge was performed three weeks respectively after the intradermal induction with 10 % test substance to the test and control group in a 24 hours exposure period. Observations on skin effects, clinical

signs and body weight were performed. The skin reactions were assessed after 48 and 72 hours.

A positive control (benzoncaine) is available from historical (May 2006) data to confirm the suitability and sensitivity of the test system and strain used. A validated method of analysis for skin sensitisation studies is not required.

Results
No mortality was observed during the study. There were no treatment related changes on the body weight development. No clinical signs were observed.

Intradermal induction with 10 % suspension of tebuconazole TC in sesame oil revealed a discrete or patchy erythema, or a moderate and confluent erythema in all 10 animals 24 h after start of exposure and a discrete or patchy erythema in 4 animals 48 h after start of exposure.

The skin was coated with SDS on the day prior to the topical induction (50 % suspension Tebuconazole) to induce skin irritation. The challenge with 10 % suspension revealed no skin reactions in any animal.

Table 6.2-18. Skin sensitisation study of tebuconazole TC

	24 hours	**48 hours**	**Total number of animals affected**
After challenge			
Tebuconazole TC	*0/10	*0/10	0
Test Vehicle Control Group	*0/5	*0/5	0
Positive control	*20/20	*20/20	20

* Number of animals with positive dermal response (scores of 1-3) /number of animals in dose group. The positive control data were obtained from the historical background of the laboratory, which was not tested concurrently, but in a separate study performed in 2006.

A historical background positive control group with 20 animals is available using benzoncaine (2 % intradermal induction, 5 % topical induction and 5 % challenge). The suitability and sensitivity of the test system and strain used has been confirmed.

Conclusion
This skin sensitisation study of tebuconazole was performed in accordance with OECD guidelines.

Under the conditions of this Guinea Pig Maximization Test according to Magnusson and Kligman, tebuconazole showed no skin-sensitising potential. No classification for skin sensitisation is required according to Regulation (EC) No. 1272/2008.

B.6.2.6.2. ***Skin sensitisation in guinea pigs (Buehler Patch Test)***

a)

Previous evaluation:	In tebuconazole DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.2.6.2/01
Study title	HWG 1608 technical - Study of skin sensitization effect on guinea pigs (Buehler Patch Test)
Test substance	(HWG 1608-technical grade) Tebuconazole
Purity (%)	97.4

Batch no.	16012/86
Test animals	Male Guinea pigs/DHPW (SPF-bred)
Groups	36 male guinea pigs (12 in test group, 12 in first control group (1st challenge), 12 in second control group (2nd challenge))
Dose	First to third induction (0.5 mL/patch, 25%, 6 hours) First challenge (0.5 mL/patch, 25%, 6 hours)
Route	Dermal
Vehicle	Distilled water containing 2 % Cremophor EL glycol
GLP	Yes
Guideline	OECD Guideline 406.
Deviation	The following deviations from the OECD-Guideline 406 (1992) occurred: - minimum of 20 test animals in treatment group not met (12 used) - Concentration used for induction did not cause mild irritation. Maximum 25% was tested in the range finding study. Question whether higher concentration should have been used
Acceptable	Acceptable
Result	Not sensitising

Methods
The skin sensitisation effect of tebuconazole was investigated in guinea pigs (Buehler Patch Test). Three groups of animals were included, one test group (12 animals) and two control groups (each 12 animals). A dose-range finding study was performed to estimate doses for the induction (highest dose which causes mild irritation – highest used in range finder was 25% which did not induce irritation) and first challenge (highest non-irritating dose). Animals were dermally treated with patches containing 25% test substance formulation (hypoallergenic dressing loaded with the test substance formulation) three times at intervals of seven days. This was the highest usable concentration. After 6 hours exposure the patches were removed and the skin was visually assessed. The animals from the control group were exposed to hypoallergenic patches moistened with physiological saline solution. The first challenge was performed 5 weeks after the dermal induction and patches containing 25% test substance formulation was applied to animals in the control and test group. Control patches were also applied to the test group. After 6 hours exposure the patches were removed. 48 and 72 hours after patch removal the skin reactions were assessed. The animals were observed for clinical signs at least once daily throughout the entire study period. The body weights of the animals were recorded before initiating the study and weekly thereafter as well as at the end of the study. A validated method of analysis for skin sensitisation studies is not required.

Results
No mortality was observed during the study. There were no treatment related changes on the body weight development. No clinical signs were observed.

Skin sensitisation
There were no skin reactions in the induction or the challenge. Because of the conclusive results of the 1st challenge, a 2nd challenge was not performed.

The sensitivity of the guinea pig strain used was verified in a separate Buehler test using formaldehyde.

Conclusion
This skin sensitisation potential of tebuconazole was investigated in a Buehler test performed in accordance with GLP and OECD/EU guidelines.

The Buehler epicutaneous patch test was performed on male guinea pigs and under the given experimental conditions, tebuconazole showed no skin-sensitising potential. No classification for skin sensitisation is required according to Regulation (EC) No. 1272/2008.

b)

Previous evaluation:	In tebuconazole DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.2.6.2/02
Study title	Dermal sensitization study with technical grade tebuconazole (Folicur) in guinea pigs

Test substance	(Folicur-technical grade) Tebuconazole
Purity (%) **Batch no.**	94.6 (active substance) 903-0133
Test animals	Male Guinea pigs/ Hartley albino (Sasco, Madison, WI)
Groups	30 male guinea pigs (15 in tebuconazole test group, 5 in tebuconazole control group), (5 in DNCB test group, 5 in DNCB control group)
Dose	First to third induction (0.4 g, 6 hours) First challenge (0.4 g, 6 hours)
Route	Dermal
Vehicle	Deionized water
GLP	Yes
Guideline	OECD Guideline 406.
Deviation	The following deviations from the OECD-Guideline 406 (1992) occurred: - minimum of 20 test animals in treatment group not met (15 used) - minimum of 10 control animal in control group not met (5 used) - DNCB used to confirm reliability/sensitivity of test is not recommended mild/moderate sensitising substance
Acceptable	Acceptable
Result	Not sensitising

Methods
The skin sensitisation effect of tebuconazole was investigated in guinea pigs (Buehler Patch Closed Patch-Technique) where methodological reliability of the test was confirmed according to current guidelines. Four groups of animals were included, one tebuconazole test group (15 animals) and one tebuconazole control group (5 animals), one DNCB test group (5 animals) and one DNCB control group (5 animals). A dose-range finding study was performed to estimate doses for the induction (highest dose which causes mild irritation) and first challenge (highest non-irritating dose). The results of the range finding study were not presented in the study report. Animals were dermally treated with patches containing 0.4 g test substance formulation three times at intervals of seven days. After 6 hours exposure the patches were removed and the skin was visually assessed. The animals from the control group were exposed to patches moistened with deionised water. The challenge was performed 4 weeks after the dermal induction and patches containing 0.4 g test substance formulation was applied to animals in the control and test group. Control patches were also applied to the test group. DCNB test and control groups were included as positive and non-induced controls. After 6 hours exposure the patches were removed. Forty-eight and 72 hours after patch removal the skin reactions were assessed. The animals were observed for clinical signs at least once daily throughout the entire study period. The body weights of the animals were recorded before initiating the study and at the end of the study. A validated method of analysis for skin sensitisation studies is not required.

Results
No mortality was observed during the study. There were no treatment related changes on the body weight development. No clinical signs were observed.

Skin sensitisation
Tebuconazole did not produce any erythema at the dose site of test- or non-induced control groups after the challenge dose (average dermal scores were 0/0 in incidence/severity). There were no skin reactions following induction or challenge. Because of the conclusive results of the 1st challenge, a 2nd challenge was not performed.

The DNCB test group showed an average dermal score of 1.0/0.9 after third induction and 1.0/1.3 after challenge. No evidence of irritation was seen at the dose site of the non-induced control group. The sensitivity and reliability of the experimental technique is therefore confirmed

Conclusion
This skin sensitisation potential of tebuconazole was investigated in a Buehler test performed in accordance with GLP and OECD/EU guidelines.

The Buehler epicutaneous patch test was performed on male guinea pigs and under the given experimental conditions, tebuconazole showed no skin-sensitising potential. No classification for skin sensitisation is required according to Regulation (EC) No. 1272/2008.

B.6.2.6.3. ***Summary of skin sensitisation studies***

Four studies investigating the skin sensitisation potential of tebuconazole have been conducted: two guinea pig maximisation tests, and two Buehler patch tests. The available studies, owned by the Bayer TF, were evaluated in the original DAR (2006), peer reviewed at EU level (EFSA Conclusion, 2008), and considered acceptable. A new skin sensitisation study (guinea pig maximisation test) has been provided for the purpose of renewal by the EU Tebuconazole Task Force. Overall, these 5 studies (3 GPMTs and 2 Buehler tests) show that tebuconazole is not a skin sensitiser and no classification according to Regulation (EC) No. 1272/2008 is required. This is consistent with the harmonised classification of tebuconazole.

B.6.2.7. Phototoxicity

An *in vitro* 3T3 NRU phototoxicity test is not required as there is no relevant absorption in the range 290 - 700 nm and the ultraviolet/visible molar extinction/absorption coefficient of the active substance is less than 10 L x mol^{-1} x cm^{-1} (see chemistry evaluation section B.2.4). Both task forces have provided justification for the non-provision of a phototoxicity study.

Bayer Taskforce
"According to the new data requirements (Commission Regulation (EU) No 283/2013 of 1 March 2013; Official Journal of the European Union, L 93/1, 3.4.2013) (1), the conduct of an *in vitro* phototoxicity study is required *"where the active substance absorbs electromagnetic radiation in the range 290-700 nm and is liable to reach the eyes or light-exposed areas of skin, either by direct contact or through systemic distribution. If the Ultraviolet/visible molar extinction/absorption coefficient of the active substance is less than 10 L x mol^{-1} x cm^{-1}, no toxicity testing is required."*
Since this coefficient is less than 10 L x mol^{-1} x cm^{-1} for tebuconazole, no toxicity testing is required."

EU Tebuconazole Task Force
No phototoxicity study is triggered, because no absorption maximum was observed above 276.5 nm (data given in the DAR) and the UV/VIS molar extinction coefficient is less than 10 L·mol^{-1}·cm^{-1}at 290 nm.

B.6.2.8. Summary of acute toxicity including irritancy and skin sensitisation

The acute toxicity of tebuconazole was investigated in multiple studies conducted via the oral, dermal and inhalation routes. Studies of skin irritancy, eye irritancy and skin sensitisation were also conducted. The available studies, owned by Bayer TF, were evaluated in the original DAR (2006), peer reviewed at EU level (EFSA Conclusion, 2008), and were considered acceptable.

In addition, the Bayer TF has provided a new study investigating the acute toxicity via the inhalation route. The EU Tebuconazole Task Force has provided new data investigating acute dermal and inhalation toxicity, plus studies of skin irritancy, eye irritancy and skin sensitisation. The new data, which are data-matching studies, support the existing EU agreed endpoints (EFSA Scientific report 2008, 176, p53). Therefore, these studies have been only briefly described in this RAR and they were not relied upon. No phototoxicity studies have been provided as testing is not triggered according to data requirements in Reg 283/2013.

The following key conclusion have been made with regards to the acute toxicity of tebuconazole:

- The results of the acute oral toxicity studies are consistent with the current EU harmonised classification of Acute Tox 4; H302 (Harmful if swallowed)
- No further classification for acute toxicity is proposed
- The data requirements of Regulation 283/2013 have been met.

Study	Species	Sex	Results	Classification Regulation (EC) No 1272/2008	Reference
Oral (Bayer Task Force)	Rat	M* F*	LD_{50}: > 5000 mg/kg bw LD_{50}: 3933 mg/kg bw	none	B.6.2.1.1/03

Oral (Bayer Task Force)	Rat	M F	LD_{50}: 4264 mg/kg bw LD_{50}: 3352 mg/kg bw	none	
Oral (Bayer Task Force)	Rat	M* only	LD_{50}: > 5000 mg/kg bw	none	B.6.2.1.1/02
Oral (Bayer Task Force)	Rat	M* F*	LD_{50}: 4000 mg/kg bw **LD_{50}: 1700 mg/kg bw**	Acute Tox 4, H302	B.6.2.1.1/01
Oral (Bayer Task Force)	Mouse	M* F*	LD_{50}: 1615 mg/kg bw LD_{50}: 3023 mg/kg bw	Acute Tox 4, H302	B.6.2.1.2/02
Oral (Bayer Task Force)	Mouse	M* F*	LD_{50}: 2800 mg/kg bw LD_{50}: 5200 mg/kg bw	none	B.6.2.1.2/01
Oral (Bayer Task Force)	Rabbit	M* F*	LD_{50}: >1000 mg/kg bw (M & F)	Acute Tox 4, H302	B.6.2.1.3/01
Dermal (Bayer Task Force) 24 hours	Rat	M F	LD_{50}: > 5000 mg/kg bw (M & F)	none	B.6.2.2/02
Dermal (Bayer Task Force) 24 hours	Rat	M F	**LD_{50}: > 2000 mg/kg bw** LD_{50}: > 2000 mg/kg bw	none	B.6.2.2/01
Dermal (EU Tebuconazole Task Force) 24 hours	Rat	M F	LD_{50}: > 2060 mg/kg bw LD_{50}: > 2060 mg/kg bw	none	B.6.2.2/03
Inhalation 1x4 hrs (aerosol) (Bayer Task Force)	Rat	M F	LC_{50}: > 0.818 mg/L LC_{50}: > 0.818 mg/L (max. attainable concentration)	none	B.6.2.3/02
Inhalation 5x6 hrs (aerosol) (Bayer Task Force)	Rat	M F	LC_{50}: > 0.24 mg/L LC_{50}: > 0.24 mg/L (max tested)	--	
Inhalation 1x4 hrs (dust, aerosol) (Bayer Task Force)	Rat	M F	**LC_{50}: > 5.093 mg/L** (dust) LC_{50}: > 0.371 mg/L (aerosol) (max. attainable concentration)	none	B.6.2.3.1/01
Inhalation 1x4 hrs (solid aerosol) (Bayer Task Force)	Rat	M F	LC_{50}: > 2.118 mg/L LC_{50}: > 2.118 mg/L (max. attainable concentration)	none	B.6.2.3/03
Inhalation (EU Tebuconazole Task Force)	Rat	M F	LC_{50} > 5.0 mg/L	none	B.6.2.6.1/03
Skin irritation	Rabbit	M	not irritating	none	B.6.2.4/01

(Bayer Task Force)		F			
Skin irritation (Bayer Task Force)	Rabbit	M F	not irritating not irritating	none	B.6.2.4/02
Skin irritation (EU Tebuconazole Task Force)	Rabbit	M only	not irritating	none	B.6.2.4/03
Eye irritation (Bayer Task Force)	Rabbit	M only	not irritating	none	B.6.2.4/01
Eye irritation (Bayer Task Force)	Rabbit	M F	mildly irritant	none	B.6.2.5.1/01
Eye irritation (EU Tebuconazole Task Force)	Rabbit	M only	mildly irritant	none	B.6.2.5.1/03
Skin sensitization (Maximization Test) (Bayer Task Force)	Guinea pigs	M only	not sensitising	none	B.6.2.6.1/02
Skin sensitization (Maximization Test) (Bayer Task Force)	Guinea pigs	F only	not sensitising	none	B.6.2.6.1/01
Skin sensitization (Buehler Test) (Bayer Task Force)	Guinea pigs	M only	not sensitising	none	B.6.2.6.2/01
Skin sensitization (Buehler Test) (Bayer Task Force)	Guinea pigs	M only	not sensitising	none	B.6.2.6.2/02
Skin sensitization (Maximization Test) (EU Tebuconazole Task Force)	Guinea pigs	M F	not sensitising	none	B.6.2.6.1/03

M = male animals
F = female animals
* = fasted animals

Based upon the results of these studies, tebuconazole is of low to moderate oral toxicity (LD_{50} 1700 mg/kg bw; clinical signs shown after acute oral administration comprised effects on the peripheral and central nervous system besides other unspecific signs of toxicity) and should be classified with acute oral category 4 (H302) under the CLP Regulation. Tebuconazole is of low acute toxicity by the dermal (LD_{50} > 200 mg/kg bw) and inhalation (4hr LC_{50} > 5.093 mg/L) routes. It is not a skin irritant and although it is slightly irritant to the eye, the CLP criteria are not met and so no classification is required. No skin sensitization was observed in Buehler patch tests or by the more sensitive Magnusson-Kligman maximisation tests. Therefore, no classification is required for acute dermal and inhalation toxicity, skin and eye irritation and skin sensitization. This is consistent with the current harmonised classification.

EU agreed acute toxicity endpoints for Tebuconazole – EFSA Scientific report	**Classification**

(2008), 176, p53)		(1272/2008)
Rat LD_{50} oral	1700 mg/kg bw (f)	Acute Tox 4, H302
Rat LD_{50} dermal	> 2000 mg/kg bw	-
Rat 4hr-LC_{50} inhalation	> 5.093 mg/L (nose only 4 h)	-
Skin irritation	Non-irritant	-
Eye irritation	Non-irritant	-
Skin sensitisation	Non-sensitiser (M&K test)	-

Phototoxicity testing is not required as the criteria in Commission Regulation (EU) No 283/2013 setting out the data requirements for active substances are not met.

B.6.3. SHORT-TERM TOXICITY

A total of eleven repeat dose toxicity studies were evaluated (nine in the original DAR (2006) and two new studies), ranging from 28-day, 90-day and 12-month studies, in a range of species including rat, mouse, rabbit, dog and cat. These studies investigated a range of routes, mainly via oral dietary (six studies) and gavage (one study) but they also included limited dermal (one study) and inhalation (three) studies.
All studies were considered to be acceptable, either as range-finding studies (supportive), specific studies (e.g. with respect to examining the cataract-inducing potential of tebuconazole) or standard studies. Range-finding studies were not conducted according to GLP or OECD test guidelines (which do not exist for range-finding studies); however standard studies were conducted according to GLP and OECD test guidelines available at the time the studies were conducted. An evaluation of deviations, as well as relevant impact of deviations, to current OECD test guidelines has been conducted for each study. No publications of relevance to short-term toxicity have been identified by the literature search.

One 28-day/4-week oral range-finding study in the rat was described in the original DAR (2006) (B.6.3.1.1/01). In addition two new 28-day/4-week oral range-finding studies in the mouse were submitted for the purpose of renewal (B.6.3.1.2/01 and B.6.3.1.2/02).

Two 90-day/13-week oral studies were described in the original DAR (2006), one in the rat (B.6.3.2.1/01) and one in the dog (B.6.3.2.2/01).

Two 12-month oral studies in the dog were described in the original DAR (2006) (B.6.3.3.1/01 and B.6.3.3.1/02).

Other routes were also investigated and described in the original DAR (2006): one 3-week dermal study in the rabbit (B.6.3.4/01), one 3-week inhalation study in the rat (B.6.3.4.2/01), and two inhalation studies to investigate cataract findings, one in the cat (B.6.3.4.3/01) and one in the dog (B.6.3.4.4/01).

B.6.3.1. Sub-acute oral studies (28-day)

B.6.3.1.1. *Study in rats*

Previous evaluation	In Tebuconazole DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.3.1.1/01
Study title	HWG 1608 – Study of the subacute oral toxicity to rats HWG 1608 – Study of the subacute oral toxicity to rats – Incidence tables of histopathological findings (Addendum to Report No. 13028)
Date	In life date: February to May 1984.
Test substance	(HWG 1608) Tebuconazole
Purity (%) **Batch no.**	97.0 16001/83
Test animals	Male and female Wistar rats, Bor:WISW (SPF-bred)
Groups	20 animals/sex/group. 10 animals of each group served as recovery groups (4 weeks recovery).
Dose (mg/kg	0, 30, 100 or 300

bw/day)	Dosing volume 10 mL/kg bw
Route	Oral, gavage
Vehicle	Aqueous suspension of 2% v/v Cremophor EL
GLP	No, but a local quality assurance procedure was followed and documented
Guideline	Methods used in this study are comparable with the OECD guideline 407 (1995) Note that the current guideline was adopted in 2008.
Deviation	Deviations from the current OECD guideline 407 (2008): No determination of food consumption performed, no assessment of the motor activity, the sensory reactivity, and the grip strength, no functional observations were conducted, no detailed clinical observation (i.e. observation in a standard arena) was performed, gaps in organ weight determination (i.e. brain and thymus), no determination of blood clotting time, gaps in clinical chemistry: sodium, potassium, total cholesterol, total protein and albumin not measured, a number of organs and tissues were not histopathologically examined: brain, spinal cord, thymus, trachea, female mammary gland, prostate, seminal vesicles with coagulating gland, peripheral nerve, skin and eyes. Historical baseline data not documented. However, motor activity, the sensory reactivity, and the grip strength, detailed functional observations, brain weight and histopathological changes of brain, eyes, spinal cord and peripheral nerves were assessed in the subchronic neurotoxicity study (B.6.7.1.2/01) at dose levels of up to 107 and 122 mg/kg bw/d in male and female rats, respectively. Food consumption, blood clotting time, cholesterol and total protein were measured in the subchronic rat study (B.6.3.2.1/01) at dose levels up to 171.7 and 235.2 mg/kg bw/d in male and female rats, respectively. In this study also the thymus, trachea, prostate, seminal vesicles and skin were histopathologically examined. All remaining missing parameters except thymus weight were assessed in the chronic rat study (B.6.5.1/01) at dose levels up to 55 and 86.3 mg/kg bw/d in male and female rats, respectively. Thymus weight was measured in the 28-day immunotoxicity study in rats (B.6.8.2.3/01).
Impact of deviations	Minor – these deviations are not considered to affect the validity of the study.
Acceptable	Acceptable
NOAEL	30 mg/kg bw/day
Effects at the LOAEL	Effects on the blood profile, increase in liver and spleen weights and sideropenia in the spleen at 100 mg/kg bw/day and above.

Methods
The methods used in this study are comparable with the OECD guideline 407 (1995) except for the deviations listed above. The administered dosages were selected on the basis of range-finding tests. The recovery groups were fed and handled exactly like the treatment groups, but were kept for an extra 4 weeks treatment-free observation period.

Results
Clinical observations
There were no treatment-related deaths in any dose group and no signs of toxicity were observed (appearance and behavioural observations) at 30 or 100 mg/kg bw/day. One control and one high-dose animal died. Alopecia occurred in all groups including control. These findings were not treatment-related. Mild lethargy was observed in a few animals of the 300 mg/kg bw/day dose group during the treatment period. In this dose group, polyuria occurred in a few animals during the 1st and 2nd weeks of treatment. Since this finding was not detected in the further course of the study and clinical chemistry and histopathology did not reveal any correlating findings, it does not appear to be toxicologically-relevant. Overall, the only treatment-related clinical sign of toxicity was mild lethargy at the top dose.

Body weight
During the 28-day treatment period, the mean body weights of the 30 and 100 mg/kg bw/day groups were comparable to those of the control group (Table 6.3-1.). In the 300 mg/kg bw/day group, statistically significant decreases in body weights (≤ 10 % change compared to control) were determined for both males and females (weeks 1 to 4) (Table 6.3-1.). In the 1st week of recovery, the rats of the highest dose group compensated for the delay in body weight gain such that they exhibited body weight gains comparable to those of the other rats; consequently at the end of the 8 week period, body weights were not statistically significantly different from

control values and % change compared to control was lowered to – 3 % and – 2 % . Overall, treatment-related effects on body weights were seen in males and females at the top dose.

Table 6.3-1. Body weight results of 28-day toxicity study in rats

Dose [mg/kg bw/day]	Males (g)				Females (g)			
	0	**30**	**100**	**300**	**0**	**30**	**100**	**300**
Week 0	164	163	163	162	157	159	156	160
(%)[a]		*(-1)*	*(-1)*	*(-1)*		*(+1)*	*(-1)*	*(+2)*
Week 1	188	184	184	**172****	167	170	164	**150****
(%)[a]		*(-2)*	*(-2)*	***(-9)***		*(+2)*	*(-2)*	***(-10)***
Week 2	215	208	208	**193****	176	178	172	**160****
(%)[a]		*(-3)*	*(-3)*	***(-10)***		*(+1)*	*(-2)*	***(-9)***
Week 3	237	229	226	**213****	179	182	177	**164****
(%)[a]		*(-3)*	*(-5)*	***(-10)***		*(+2)*	*(-1)*	***(-8)***
Week 4	253	245	246	**227****	184	188	184	**174****
(%)[a]		*(-3)*	*(-3)*	***(-10)***		*(+2)*	*(±0)*	***(-5)***
Week 8	298	288	294	289	198	204	195	194
(%)[a]		*(-3)*	*(-1)*	*(-3)*		*(+3)*	*(-2)*	*(-2)*

(%)[a] % change compared to control
* statistically significant difference from control $p \leq 0.05$
** statistically significant difference from control $p \leq 0.01$

Haematology and clinical chemistry

Treatment group: At 100 and 300 mg/kg bw/day, the haematocrit values of both sexes were significantly lower (≤10 % change compared to control in males but > 10 % in females at 300 mg/kg bw/day) (Table 6.3-2). In 300 mg/kg bw/day males, erythrocyte count and the haemoglobin content were reduced (≤ 10 % change compared to control). The slight decrease in the erythrocyte count was attributed to the very low value of one animal and thus is not toxicologically relevant. In 100 and 300 mg/kg bw/day females the haemoglobin content and MCV were statistically significantly decreased (≤10 % change compared to control at 100 mg/kg bw/day but > 10 % at 300 mg/kg bw/day) and in 300 mg/kg bw/day females the MCH was decreased (>10 % change compared to control) (Table 6.3-2). In the 300 mg/kg bw/day females, a higher leucocyte count in comparison to the control was observed (73.3 % change compared to control). Overall, there were adverse and treatment-related effects on some haematological parameters in males and females from 100 mg/kg bw/day.

Table 6.3-2. Haematology data of 28-day toxicity study

Dose [mg/kg bw/day]	Males				Females			
	0	**30**	**100**	**300**	**0**	**30**	**100**	**300**
Erythrocytes [Tera/L]	8.50	8.16	8.15	7.93*	8.02	7.77	7.88	7.94
(%)[a]	-	*(-4.0)*	*(-4.1)*	*(-6.7)*	-	*(-3.1)*	*(-1.7)*	*(-1.0)*
Haemoglobin [g/L]	165	163	159	153*	157	152	147**	136**
(%)[a]	-	*(-1.2)*	*(-3.6)*	*(-7.3)*	-	*(-3.2)*	*(-6.4)*	*(-13.4)*
Haematocrit [L/L]	0.50	0.49	0.48*	0.46**	0.47	0.46	0.44**	0.41**
(%)[a]	-	*(-2.0)*	*(-4.0)*	*(-8.0)*	-	*(-2.1)*	*(-6.4)*	*(-12.8)*
MCV [FL]	59	60	59	59	58	59	56*	52**
(%)[a]	-	*(1.7)*	*(0.0)*	*(0.0)*	-	*(1.7)*	*(-3.4)*	*(-10.3)*
MCH [pg/ery]	19.5	20.0	19.6	19.4	19.6	19.6	18.7	17.1**
(%)[a]	-	*(2.6)*	*(0.5)*	*(-0.5)*	-	*(0.0)*	*(-4.6)*	*(-12.8)*
Leucocytes [Giga/L]	8.0	7.8	6.8	9.4	6.0	5.6	6.0	10.4**
(%)[a]	-	*(-2.5)*	*(-15.0)*	*(17.5)*	-	*(-6.7)*	*(0.0)*	*(73.3)*
Reticulocytes [0/00]	16	14	14	17	14	13	19	21*
(%)[a]	-	*(-12.5)*	*(-12.5)*	*(6.3)*	-	*(-7.1)*	*(35.7)*	*(50.0)*

(%)[a] % change compared to control
* statistically significant difference from control $p \leq 0.05$
** statistically significant difference from control $p \leq 0.01$

Recovery group: The haematological findings obtained at the end of the observation period without treatment

were normal and no toxicologically relevant differences were found in the dose groups in comparison with the controls.

The leukocyte differential count also revealed no anomalies.

Treatment group: In clinical chemistry measurements, no effects were seen at 30 and 100 mg/kg bw/day. At 300 mg/kg bw/day, the ASAT and ALAT activities of the male rats were slightly higher and the ASAT, ALAT, and ALP activities of the female rats were markedly increased (> 10 % change compared to control); this is indicative of liver damage. Changes in glucose and urea concentrations and other changes were not dose-related and thus are regarded as normal variation (Table 6.3-3.). Overall, treatment-related effects in some clinical-chemistry parameters (mainly indicative of liver damage) were seen at the top dose.

The analyses of the urine and the urine sediment did not reveal findings deviating from the norm or dose-related differences between the animals of the control and dose groups.

Table 6.3-3. Clinical chemistry data of 28-day toxicity study

Dose [mg/kg bw]	**Week**	**Males**				**Females**			
		0	**30**	**100**	**300**	**0**	**30**	**100**	**300**
ASAT [U/L]	4	61.7	54.8	56.0	74.5	50.6	56.9	65.5	144.6**
	8	59.8	53.4	58.6	60.0	63.4	51.7	72.7	59.5
ALAT [U/L]	4	55.4	52.8	49.4	74.4	35.7	39.9	42.4	120.1**
	8	59.5	53.6	55	49.4	46.6	36.5	44.2	42.1
ALP [U/L]	4	435	368	371	394	192	191	178	544**
	8	261	277	284	240	156	162	152	188
Urea [mmol/L]	4	7.49	7.49	7.01	7.04	6.61	9.44**	7.46	8.78
	8	8.34	8.20	7.48	7.47**	7.72	8.60	7.59	8.95
Glucose [mmol/L]	4	5.45	5.69	6.51**	6.05	6.24	5.87	5.91	5.42**
	8	5.38	5.76	5.66	5.77	5.68	5.76	5.52	5.64
Creatinine [µmol/L]	4	65	57	54	49**	54	53	56	53
	8	74	64**	69	58**	70	68	67	70

ALAT: Alanine aminotransferase ASAT: Aspartate aminotransferase ALP: Alkaline phosphatase
* statistically significant difference from control $p \leq 0.05$
** statistically significant difference from control $p \leq 0.01$

Recovery group: The values determined at the end of the observation period were normal and there were no toxicologically relevant differences between the dose groups and the control group. The slightly lower values for ASAT, urea, and creatinine in some treatment groups are toxicologically irrelevant.

The analyses of the urine and the urine sediment at the end of the observation period revealed no treatment-related findings.

Treatment group: The liver microbial enzyme measurements show that 100 and 300 mg/kg bw/day resulted in induction of these enzyme systems. Statistically significant increases (> 10 % up to +124.5 % change compared to controls) were found for all examined microbial enzymes in the rats at the highest dose. At 100 mg/kg bw/day, the N- and O-demethylase activities in the males were still statistically significantly increased (> 10 % change compared to control), and in the females they were higher than those of the control animals (> 10 % change compared to control). The results for the 30 mg/kg bw/day groups did not show statistically relevant increases in comparison to control but did show (> 10 % change compared to control). In addition, the male rats of the highest dose group had a higher triglyceride concentration in the liver tissue (> 50 % change compared to control) (Table 6.3-4.). This is consistent with the ADME data which revealed that that tebuconazole is efficiently metabolised, leaving hardly any unchanged parent compound in excreta 72 h after administration (B.6.1.3.). Overall, liver enzyme induction was observed from 100 mg/kg bw/day.

Table 6.3-4. Liver enzyme induction data of 28-day toxicity study

Dose [mg/kg bw/day]	Males				Females			
	0	**30**	**100**	**300**	**0**	**30**	**100**	**300**
N-demethylase [nmol/g/min]	152.6	170.8	220.9*	205.5*	60.0	70.7	81.5	128.2**
(%)[a]	-	*(11.9)*	*(44.8)*	*(34.7)*	-	*(17.8)*	*(35.8)*	*(113.7)*
O-demethylase [nmol/g/min]	9.1	11.6	14.3**	19.4**	8.8	10.2	11.0	14.1**
(%)[a]	-	*(27.5)*	*(57.1)*	*(113.2)*	-	*(15.9)*	*(25.0)*	*(60.2)*
Cytochrome P-450 [nmol/g]	29.8	40.0	35.3	66.9**	29.6	33.1	33.5	55.3**
(%)[a]	-	*(34.2)*	*(18.5)*	*(124.5)*	-	*(11.8)*	*(13.2)*	*(86.8)*
Triglycerides [µmol/g]	5.22	4.52	6.49	8.33**	6.52	6.29	6.15	6.53
(%)[a]	-	*(-13.4)*	*(24.3)*	*(59.6)*	-	*(-3.5)*	*(-5.7)*	*(0.2)*

* statistically significant difference from control $p \leq 0.05$
** statistically significant difference from control $p \leq 0.01$
(%)[a] percent change relative to control

Recovery group: There were no treatment-related changes in liver enzyme induction at the end of the recovery period.

Gross pathology and histopathology
Treatment group: No treatment-related changes were found during gross examination of the experimental animals at the end of the treatment phase.

Absolute and relative mean liver weights of the male and female rats at 100 and 300 mg/kg bw/day were increased (Table 6.3-5); these increases were statistically significant for relative liver weight in males and females (≤10 % change relative to control at 100 mg/kg bw/day and > 10 % change relative to control at 300 mg/kg bw/day) but was only statistically significant for absolute liver weight for females at 300 mg/kg bw/day (> 10 % change compared to control). Liver weight increases (both absolute and relative) occurred in a dose dependent manner, these findings accompany liver enzyme induction, were associated with histopathological findings and were observed in both sexes. Consequently they are considered to be treatment-related and adverse from the dose level of 100 mg/kg bw/day in both males and females. There were increases in the relative mean spleen weight for the male rats of the highest dose group (17.9 % change compared to control) and in the mean absolute and relative spleen weight for the female rats of the intermediate and highest dose groups (> 10 % change compared to control) (Table 6.3-5). Spleen weight increased (both absolute and relative) occurred in a dose dependent manner in females only, this was accompanied with histopathological findings in males of the top dose group and females of the mid and top dose group. The females of the intermediate and highest dose groups also showed an increased mean kidney weight, this was statistically significant for relative kidney weight in females at both 100 and 300 mg/kg bw/day and for absolute kidney weight in females at 300 mg/kg bw/day (top dose) only. In male rats, however, a decrease was observed, so that the kidney weight change in the female animals is not regarded as treatment-related. Other differences in mean organ weights were not dose-related and were likely normal variation. Overall, adverse and treatment-related effects on the weights of liver and spleen were seen from 100 mg/kg bw/day.

Table 6.3-5. Selected organ weight data (absolute and relative weights) of the 28-day toxicity study

Dose [mg/kg bw/day]	Males				Females			
	0	**30**	**100**	**300**	**0**	**30**	**100**	**300**
Liver, absolute [mg]	9905	9796	10375	11122	6494	6750	7262	9594**
(%)[a]	-	*-1.1*	*4.7*	*12.3*	-	*3.9*	*11.8*	*47.7*
Liver, relative [mg/100 g]	3899	3999	4209*	5029**	3562	3657	3946*	5447**
(%)[a]	-	*2.6*	*8.0*	*29.0*	-	*2.7*	*10.8*	*52.9*

Dose [mg/kg bw/day]	Males				Females			
	0	30	100	300	0	30	100	300
Spleen, absolute [mg]	495	458	481	509	371	393	460**	541**
(%)[a]	-	*-7.5*	*-2.8*	*2.8*	-	*5.9*	*24.0*	*45.8*
Spleen, relative [mg/100 g]	195	187	195	230	203	213	250**	306**
(%)[a]	-	*-4.1*	*0.0*	*17.9*	-	*4.9*	*23.2*	*50.7*
Kidney, absolute [mg]	1626	1507	1565	1371**	1106	1121	1195	1273**
(%)[a]	-	*-7.3*	*-3.8*	*-15.7*	-	*1.4*	*8.0*	*15.1*
Kidney, relative [mg/100 g]	641	615	635	622	606	608	650*	724**
(%)[a]	-	*-4.1*	*-0.9*	*-3.0*	-	*0.3*	*7.3*	*19.5*

* statistically significant difference from control $p \leq 0.05$
** statistically significant difference from control $p \leq 0.01$
(%)[a] percent change relative to control

In 300 mg/kg bw/day females, an increase in connective tissue fibers was detected in the spleen in the region of the red pulp (sclerosis of the pulp), which was associated with a decrease in the iron content (sideropenia). Sideropenia was also seen in the top dose males and in the 100 mg/kg bw/females. The splenic follicles appeared to be atrophied. In the liver of the top dose females, there was an increase in periportal stroma, with occasional histiocytes and leukocytes. Also, bile duct proliferation was clearly observed. In the hepatocytes, variable-sized droplet fatty change was observed at the top dose in both sexes. In some females at the top dose, the hepatocellular mitotic rate appeared to be increased. In the lungs of two top dose females the endothelial cells in a few blood vessels were severely proliferated and occasionally resulted in vascular occlusions. The cytoplasm of the proliferated endothelial cells sometimes had a foamy-honeycombed structure. In the adrenals of the top dose females, the cells of the zona fasciculata (cortex) were irregularly arranged and contained increased variable-sized (fat) vacuoles. A similar finding was seen in several repeat dose toxicity studies – in rats (8-week and 13-week studies), in mice (8-week study), and in dogs (13-week and 1-year studies); this observation is therefore likely to be a treatment-related effect. In males at the top dose, there was enlargement of the zona glomerulosa of the adrenal cortex in the bone marrow of two top dose females, an increased occurrence of fat cells was found, which resulted in a reduction in haematopoiesis in this tissue section. Overall, treatment-related histopathological effects were seen at the top dose in the spleen, liver and adrenal of both sexes and in lungs of females.

Table 6.3-6. Incidences of histopathological findings in the 28-day toxicity study

Dose [mg/kg bw]	Males				Females			
	0	30	100	300	0	30	100	300
Liver								
Number of animals examined	5	./.	5	5	4	./.	5	5
Centrilobular hepatocytes enlarged								
Total	0/5	./.	0/5	5/5	0/4	./.	0/5	0/5
Fatty change in hepatocytes								
Minimal	0	./.	0	0	0	./.	0	1
Mild	0	./.	5	5	1	./.	0	2
Moderate	0	./.	0	0	0	./.	0	0
Severe	0	./.	0	0	0	./.	0	0
Total	0/5	./.	5/5	5/5	1/4	./.	0/5	3/5
Hepatocellular mitoses increased								
Total	0/5	./.	0/5	0/5	0/4	./.	0/5	2/5 Mi
Periportal stroma increased, Bile duct proliferation								
Minimal	0	./.	0	0	0	./.	0	0
Mild	0	./.	0	0	0	./.	0	0
Moderate	0	./.	0	0	0	./.	0	5
Severe	0	./.	0	0	0	./.	0	0
Total	0/5	./.	0/5	0/5	0/4	./.	0/5	5/5
Spleen								
Number of animals examined	5	./.	5	5	4	5	5	5
Reduced iron content								
Minimal	0	./.	0	5	0	0	0	0

Dose [mg/kg bw]	Males				Females			
	0	30	100	300	0	30	100	300
Mild	0	./.	0	0	0	0	0	0
Moderate	0	./.	0	0	0	0	1	0
Severe	0	./.	0	0	0	0	4	5
Total	0/5	./.	0/5	5/5	0/4	0/5	5/5	5/5
Increased no. of reticular fibres (sclerosis of pulp)								
Minimal	0	./.	0	0	0	0	0	0
Mild	0	./.	0	0	0	0	0	0
Moderate	0	./.	0	0	0	0	0	0
Severe	0	./.	0	0	0	0	0	5
Total	0/5	./.	0/5	0/5	0/5	0/5	0/5	5/5
Adrenals								
Number of animals examined	5	./.	5	5	4	./.	5	5
Enlargement of zona glomerulosa in adrenal cortex								
Total	0/5	./.	0/5	4/5	0/5	./.	0/5	0/5
Irregularly arranged fasciculate cells/changes sinus endothelial cells								
Minimal	0	./.	0	0	0	./.	0	0
Mild	0	./.	0	0	0	./.	0	0
Moderate	0	./.	0	0	0	./.	0	1
Moderate to severe	0	./.	0	0	0	./.	0	4
Total	0/5	./.	0/5	0/5	0/5	./.	0/5	5/5
Lung								
Number of animals examined	5	./.	5	5	4	./.	5	5
Proliferated endothelium of blood vessels								
Total	0/5	./.	0/5	0/5	0/4	./.	0/5	2/5
Bone marrow								
Number of animals examined	5	./.	./.	5	4	./.	5	5
Increased occurrence of yellow marrow								
Minimal	0	./.	./.	0	0	./.	0	0
Mild	0	./.	./.	0	0	./.	0	0
Moderate	0	./.	./.	0	0	./.	0	2
Severe	0	./.	./.	0	0	./.	0	0
Total	0/5	./.	./.	0/5	0/5	./.	0/5	2/5

./. = no data given in report
Mi = hepatocellular mitoses appear to occur in increased amounts (which indicates a qualitative assessment)

Recovery group: Gross examination of the experimental animals from the recovery groups at the end of the observation period did not reveal any abnormal finding and the organ weights did not show any treatment-related changes.

After the recovery period, in the top dose females, an increase in fibre content was observed in the red pulp of the spleen and in the periportal fields of the liver. The zona fasciculata of the adrenals of the females exhibited mild reactions of the sinus endothelial cells and (fat) vacuoles. In the other dose groups, no histopathological changes attributable to treatment with the test compound were detected. An overview is given in the following table.

Table 6.3-7. Incidences of histopathological findings in the 28-day toxicity study after the recovery period

Dose [mg/kg bw]	Males				Females			
	0	30	100	300	0	30	100	300
Liver								
Number of animals examined	5	./.	./.	5	5	./.	5	5
Periportal stroma increased, Bile duct proliferation								

Dose [mg/kg bw]	Males				Females			
	0	**30**	**100**	**300**	**0**	**30**	**100**	**300**
Minimal	0	./.	./.	0	0	./.	0	0
Mild	0	./.	./.	0	0	./.	0	4
Moderate	0	./.	./.	0	0	./.	0	1
Severe	0	./.	./.	0	0	./.	0	0
Total	0/5	./.	./.	0/5	0/5	./.	0/5	5/5
Spleen								
Number of animals examined	5	./.	./.	5	5	./.	5	5
Increased no. of reticular fibres (sclerosis of pulp)								
Minimal	0	./.	./.	0	0	./.	0	0
Mild	0	./.	./.	0	0	./.	0	0
Moderate	0	./.	./.	0	0	./.	0	5
Severe	0	./.	./.	0	0	./.	0	0
Total	0/5	./.	./.	0/5	0/5	./.	0/5	5/5
Adrenals								
Number of animals examined	5	./.	./.	5	5	./.	5	5
Irregularly arranged fasciculate cells/changes sinus endothelial cells								
Minimal	0	./.	./.	0	0	./.	0	4
Mild	0	./.	./.	0	0	./.	0	1
Moderate	0	./.	./.	0	0	./.	0	0
Severe	0	./.	./.	0	0	./.	0	0
Total	0/5	./.	./.	0/5	0/5	./.	0/5	5/5
Lung								
Number of animals examined	./.	./.	./.	./.	5	./.	./.	5
Proliferated endothelium of blood vessels								
Minimal	./.	./.	./.	./.	0	./.	./.	0
Mild	./.	./.	./.	./.	0	./.	./.	0
Moderate	./.	./.	./.	./.	0	./.	./.	0
Severe	./.	./.	./.	./.	0	./.	./.	0
Total	./.	./.	./.	./.	0/5	./.	./.	0/5

Conclusion
In this 28-day study in rats, mild lethargy, effects on body weights and treatment-related effects in some clinical-chemistry parameters (mainly indicative of liver damage) were seen at the top dose of 300 mg/kg bw/day. In addition, there were adverse and treatment-related effects on some haematological parameters and on the weights of liver and spleen from 100 mg/kg bw/day. Liver enzyme induction was also observed from 100 mg/kg bw/day. Histopathological effects were seen at the top dose in the spleen, liver and adrenal of both sexes and in lungs of females. Effects on the spleen (sideropenia) were also seen in females at 100 mg/kg bw/day.

The effects on body weight, haematology, clinical chemistry and enzyme activity were reversible at the end of the recovery period, whereas some histopathological findings , in the liver, spleen and adrenals of females, were still seen.

Overall, a LOAEL was established at 100 mg/kg bw/day in this study based on effects on some haematological parameters, changes in the weight of liver and spleen and sideropenia in females. A NOAEL for both sexes was established in this study at 30 mg/kg bw/day. This is the same NOAEL value agreed during the first review of tebuconazole.

B.6.3.1.2. ***Studies in mice***

Two new studies are available.

a)

Previous evaluation	None: Submitted for the purpose of renewal.

	(study owned by Bayer Task force)

Study ID	B.6.3.1.2/01
Study title	HWG 1608 - Range-finding toxicological study with NMRI mice to establish dosage for a chronic study (feeding for four weeks)
Dates	In-life dates: 11.9.1984 – 9.10.1984
Test substance	HWG 1608, Tebuconazole (technical grade)
Purity (%) **Batch no.**	96.9 Mixed batch with fl. No. 132
Test animals	Mouse Bor: NMRI (SPF Han)
Groups	5/sex/dose
Dose (ppm)	0, 125, 500 and 2000
Route	Oral, diet
Vehicle	Basal diet, no positive control
GLP	No.
Guideline	Not applicable, there are no OECD guidelines for range-finding studies.
Deviation	Since this study was a dose range finding study, and no OECD guideline was cited no statement on deviations can be given.
Acceptable	Acceptable as a supporting study only.
Proposed doses to be taken forward	Doses of 0, 20, 60 and 180 ppm were proposed for the chronic toxicity feeding study in mice.

Methods
Groups of five male and five female NMRI mice were administered tebuconazole at doses of 0 (control), 125, 500 and 2000 ppm per day in the diet (doses in mg/kg bw/day given in Table 6.3-8.) for four weeks. Animals were observed twice daily and recordings were made (at the start of the study and then weekly) of body weight and food intake. A clinical examination of all animals was carried out at the end of the study. At the end of the study all surviving animals were sacrificed and the following organs were weighed: heart, testicles, ovaries, liver, lung, spleen, kidneys and adrenals. Animal livers in all groups were histopathologically examined, and in the case of controls and the high dose group also the kidneys, lung, stomach, intestines, pancreas, urinary bladder and thyroid, and sporadically seminal vesicle, testicles, epididymis and prostate.

Table 6.3-8. Study design and doses

Test group		**1**	**2**	**3**	**4**
Concentration in diet	[ppm]	0	125	500	2000
Dose per animal [mg/kg bw/day]	Male	0.0	12.6	47.0	181.7
	Female	0.0	14.1	53.2	236.0

Results
Clinical observations
No treatment-related deaths or effects on the appearance and behaviour of the mice were observed. Some animals across all groups died, most likely as a consequence of the blood sampling procedure.

Body weight and food intake
Male and female animals in the 2000 ppm group lost weight (males lost 2 g and females lost 1 g) during the first study week. This may have been related to the reduction in food consumption in males (-12 % compared to control) and females (-9 % compared to control) during the first week. By the end of the study, female bodyweights in the mid-dose group (500 ppm) was lower (-11 %) compared to controls and bodyweight gain was also lower (-75 %) compared to controls. By the end of the study, female bodyweights in the high-dose group (2000 ppm) increased to a level similar to that of the controls, however bodyweight gain was still lower (-50 %) compared to controls. Male bodyweights in the low-dose group were unchanged compared to controls. In the mid-dose group (500 ppm), by the end of the study, male bodyweight were only slightly reduced (- 3 %) compared to controls, however bodyweight gain was greatly reduced (- 43 %) compared to controls. Males in the high-dose group (2000 ppm) gained weight after the first study week (day 8 onwards), but their weights remained below those of the controls during the rest of the study and bodyweight gain was also greatly reduced (- 43 %) compared to controls.

Overall, changes in bodyweights were not seen at levels regarded as adverse, however, significant effects on bodyweight gain (> 10 % compared to control) were seen in males and females from 500 ppm.

Food and water consumption was generally reduced across the four week period, in males and females, in all treated groups. While no consistent dose-related pattern was evident in the reduction in food and water consumption, a clear reduction in food consumption during week 4 was evident (-26 % to -88 % compared to controls). Therefore the reduction in food consumption may be related to the general health of the animals, rather than a palatability issue.

Table 6.3-9. Body weights (g) (and % difference to control)

	Tebuconazole (ppm)						
	0	**125**	***(%)[a]***	**500**	***(%)[a]***	**2000**	***(%)[a]***
Males							
Day 1	25	25	*(±0)*	27	*(+8)*	26	*(+4)*
Day 8	28	28	*(±0)*	29	*(+4)*	24	*(-14)*
Day 15	30	30	*(±0)*	30	*(±0)*	29	*(-3)*
Day 22	31	31	*(±0)*	31	*(±0)*	29**	*(-6)*
Day 28	32	32	*(±0)*	31	*(-3)*	30**	*(-6)*
Body weight gain day 1-28	7	7	*(±0)*	4	*(-43)*	4	*(-43)*
Females							
Day 1	23	24	*(+4)*	23	*(±0)*	24	*(+4)*
Day 8	24	25	*(+4)*	23	*(-4)*	23	*(-4)*
Day 15	25	26	*(+4)*	23	*(-8)*	25	*(±0)*
Day 22	26	26	*(+0)*	24	*(-8)*	26	*(±0)*
Day 28	27	27	*(+0)*	24	*(-11)*	26	*(-4)*
Body weight gain day 1-28	4	3	*(-25)*	1	*(-75)*	2	*(-50)*

[a] *% difference compared to control*

* $p \leq 0.05$; ** $p \leq 0.01$

Table 6.3-10. Feed consumption (mg/kg bw/d) (and % difference to control)

	Tebuconazole (ppm)						
	0	**125**	***(%)[a]***	**500**	***(%)[a]***	**2000**	***(%)[a]***
Males							
Week 1	118	113	*(-4)*	110	*(-7)*	104	*(-12)*
Week 2	124	116	*(-6)*	101	*(-19)*	132	*(+6)*
Week 3	119	118	*(-1)*	113	*(-5)*	113	*(-5)*
Week 4	118	55	*(-53)*	52	*(-56)*	14	*(-88)*
Females							
Week 1	122	121	*(-1)*	121	*(-1)*	111	*(-9)*
Week 2	125	125	*(±0)*	122	*(-2)*	137	*(+10)*
Week 3	127	129	*(+2)*	138	*(+9)*	125	*(-2)*
Week 4	132	77	*(-42)*	88	*(-33)*	98	*(-26)*

[a] *% difference compared to control*

No statistical analysis performed

Table 6.3-11. Water consumption (g/kg bw/d) (and % difference to control)

	Tebuconazole (ppm)						
	0	**125**	***(%)[a]***	**500**	***(%)[a]***	**2000**	***(%)[a]***
Males							
Week 1	253	231	*(-9%)*	211	*(-17%)*	171	*(-23%)*
Week 2	266	236	*(-11%)*	205	*(-23%)*	233	*(-12%)*
Week 3	225	206	*(-8%)*	197	*(-12%)*	163	*(-28%)*
Week 4	305	263	*(-14%)*	241	*(-21%)*	269	*(-12%)*

	Tebuconazole (ppm)						
	0	**125**	***(%)[a]***	**500**	***(%)[a]***	**2000**	***(%)[a]***
Females							
Week 1	274	269	*(-2%)*	236	*(-14%)*	214	*(-22%)*
Week 2	288	212	*(-26%)*	171	*(-41%)*	272	*(-6%)*
Week 3	255	265	*(+4%)*	245	*(-4%)*	238	*(-7%)*
Week 4	356	336	*(-6%)*	362	*(+2%)*	345	*(-3%)*

[a] *% difference compared to control*
No statistical analysis performed

Haematology and clinical chemistry
No treatment-related effects on the measured haematological parameters were noted.

In clinical chemistry, in both sexes transaminase activity was markedly increased at 2000 ppm, and in females also at 500 ppm (> 10 % change compared to control) (Table 6.3-12.). The alkaline phosphatase (AP) activity was increased (statistically significantly increased in males only) from 500 ppm onwards (> 10 % change compared to control). Cholesterol was reduced (> 10 % compared to controls) in both sexes in all treatment groups and was statistically significantly reduced in females from 500 ppm. The observed changes in phosphate concentration did not correlate with the dose and were therefore not regarded as toxicologically-relevant. Overall, there were adverse, treatment-related effects on some clinical-chemistry parameters from 500 ppm.

Table 6.3-12. Clinical chemistry data of 28-day toxicity study in mice

Dose [ppm]	**Males**				**Females**			
	0	**125**	**500**	**2000**	**0**	**125**	**500**	**2000**
ASAT [U/L]	44.1	40.3	46.4	67.8*	37.2	57.5	59.4**	162.9**
(%)[a]	-	*(-8.6)*	*(+5.2)*	*(+53.7)*	-	*(+54.6)*	*(+59.7)*	*(+337.9)*
ALAT [U/L]	71.4	51.3	35.7	113.2	34.2	48.0	68.6*	315.4**
(%)[a]	-	*(-28.2)*	*(-50.0)*	*(+58.5)*	-	*(+40.4)*	*(+100.6)*	*(+822.2)*
Cholesterol [mmol/L]	4.17	3.62	2.58	1.29	3.44	2.81	1.54*	1.07*
(%)[a]	-	*(-13.2)*	*(-38.1)*	*(-69.1)*	-	*(-18.3)*	*(-55.2)*	*(-68.9)*
AP [U/L]	162	178	214**	293*	239	217	325	295
(%)[a]	-	*(+9.9)*	*(+32.1)*	*(+80.9)*	-	*(-9.2)*	*(+36.0)*	*(+23.4)*
P [mmol/L]	2.60	2.44	2.57	1.94*	2.57	2.64	1.75*	2.24
(%)[a]	-	*(-6.2)*	*(-1.2)*	*(-25.4)*	-	*(+2.7)*	*(-31.9)*	*(-12.8)*

ALAT: Alanine aminotransferase ASAT: Aspartate aminotransferase
AP: Alkaline phosphatase P: Phosphate
* statistically significant difference from control p≤0.05
** statistically significant difference from control p≤0.01
(%)[a] percent change compared to control

Gross pathology and histopathology
No toxicologically relevant gross findings were found at necropsy. The absolute and relative liver weights of the 2000 ppm males were increased (> 15 % change compared to control). In females the absolute liver weights were statistically significantly increased from 500 ppm and above (> 15 % change compared to control), and relative liver weight at all doses (> 15 % change compared to control) (Table 6.3-13.). The other organ weights were not affected by treatment. Overall, liver weight was increased in females from 125 ppm and in males at the top dose.

Table 6.3-13. Organ weight data of 28-day toxicity study in mice

Dose [ppm]	**Males**				**Females**			
	0	**125**	**500**	**2000**	**0**	**125**	**500**	**2000**
Liver, absolute [mg]	1765	1701	1935	2718*	1267	1507	1509*	2065**
(%)[a]	-	*(-3.6)*	*(+9.6)*	*(+54.0)*	-	*(+18.9)*	*(+19.1)*	*(+63.0)*

Dose [ppm]	Males				Females			
	0	**125**	**500**	**2000**	**0**	**125**	**500**	**2000**
Liver, relative [mg/100 g]	5459	5339	6227	8998*	4817	5669*	6281**	8045**
(%)[a]	-	*(-2.2)*	*(+14.1)*	*(+64.8)*	-	*(+17.7)*	*(+30.4)*	*(+67.0)*

* statistically significant difference from control p≤0.05
** statistically significant difference from control p≤0.01
(%)[a] percent change compared to control

In all animals of the 2000 and 500 ppm dose groups and in one male and one female of the 125 ppm group, hepatic lipid accumulation in the hepatocyte plasma was observed. Some (female) mice exhibited an increased content of 'double refractile' lipids. Due to the lipid accumulation in the hepatocytes a no-effect dose could not be established from a histological viewpoint.

Table 6.3-14. Incidences of histopathological findings in the 28-day toxicity study in mice

Dose [ppm]	Males				Females			
	0	**125**	**500**	**2000**	**0**	**125**	**500**	**2000**
Liver								
Number of animals examined	1	5	5	4	0	5	5	4
Lipid accumulation in the hepatocyte plasma								
Slight, few small	0	1	0	0	0	0	0	0
Slight, few small - Moderate	0	0	5	0	0	1	5	0
Moderate - Severe	0	0	0	4	0	0	0	4
Total	0/1	1/5	5/5	4/4	0/0	1/5	5/5[a]	4/4[b]
Necrosis	1	0	0	0	0	0	0	0
Bile duct cyst(s)	1	0	0	0	0	0	0	0
Cellular/inflammatory cellular infiltrates	1	0	0	0	0	0	0	0
Stomach								
Number of animals examined	5	ne	ne	3	5	ne	ne	5
Normal	5	ne	ne	3	5	ne	ne	5
Intestines								
Number of animals examined	5	ne	ne	3	5	ne	ne	5
Normal	5	ne	ne	3	5	ne	ne	5
Pancreas								
Number of animals examined	4	ne	ne	3	1	ne	ne	2
Normal	4	ne	ne	3	1	ne	ne	2
Urinary bladder								
Number of animals examined	5	ne	ne	3	5	ne	ne	5
Normal	5	ne	ne	3	5	ne	ne	5
Thyroid								
Number of animals examined	5	ne	ne	4	5	ne	ne	5
Normal	5	ne	ne	4	5	ne	ne	5
Seminal vesicle								
Number of animals examined	ne	ne	ne	4	-	-	-	-
Normal	ne	ne	ne	4	-	-	-	-

ne = not examined
[a] light-refractive lipids (3/5)
[b] light-refractive lipids (2/4)

Conclusion
In this limited range-finding 28-day dietary study in mice there were no treatment-related effects on the appearance and behaviour of the animals, and there were no treatment-related deaths. Food and water intake generally reduced across the four week period, in males and females, in all treated groups. Body weight gain was reduced from 500 ppm in both males and females.

There were treatment-related effects on some clinical-chemistry parameters from 500 ppm. Statistically significantly increased transaminases and alkaline phosphatase activities in both sexes were recorded from

500 ppm (equivalent to 47 and 53 mg/kg bw/day in males and females respectively) and above. Cholesterol was reduced in both sexes at 500 and 2000 ppm. The main target tissue was the liver. Absolute and relative liver weights were increased at 2000 ppm in males (equivalent to 181 mg/kg bw/day) and from 125 ppm and above in females (equivalent to 14 mg/kg bw/day). Gross pathological examination revealed pale and partly patchy livers at 500 ppm (in females only) and at 2000 ppm (in both sexes). An increased lipid accumulation in the hepatocytes from 125 ppm (equivalent to 13 and 14 mg/kg bw/day in males and females respectively – the lowest dose) and above was seen; therefore a NOAEL was not established. The overall LOAEL was 125 ppm (equivalent to 13 and 14 mg/kg bw/day in males and females respectively).

Based on this study, doses of 0, 20, 60 and 180 ppm were proposed for the chronic toxicity feeding study in mice (Section B.6.5.1, B.6.5.2/01).

b)

Previous evaluation	None: Submitted for the purpose of renewal. (study owned by Bayer Task force)

Study ID	B.6.3.1.2/02
Study title	HWG 1608 - Range-finding toxicological study with NMRI mice to establish dosage for a chronic study (feeding for eight weeks) and for determination of enzyme induction in the liver (feeding for five days)
Dates	In-life dates: 25.10 - 18.12.1984
Test substance	HWG 1608, Tebuconazole (technical grade)
Purity (%) **Batch no.**	96.9 Mixed batch with fl. No. 132
Test animals	Mouse Bor: NMRI (SPF Han)
Groups	5/sex/dose
Dose (ppm)	0, 500 and 2000
Route	Oral, diet.
Vehicle	Basal diet, no positive control
GLP	No
Guideline	Not applicable, there are no OECD guidelines for range-finding studies.
Deviation	Since this study was a dose range finding study, and no OECD guideline was cited, no statement on deviations can be given.
Acceptable	Acceptable as a supporting study only.
Proposed doses to be taken forward	Doses of 0, 20, 60 and 180 ppm were planned for the oncogenicity study in mice.

Methods
Groups of five male and female NMRI mice were administered tebuconazole for eight weeks at the doses of 0, 500 and 2000 ppm in their diet (doses in mg/kg bw/day given in Table 6.3-15)

Table 6.3-15. Study design and doses

Test group		**1**	**2**	**3**
Concentration in diet	[ppm]	0	500	2000
Dose per animal [mg/kg bw/day]	Male	0	82*	329*
	Female	0	114*	454*

** extrapolated values; values for daily food intake (g/kg bw) taken from wk 1-8 in 21-months dietary study (Bomhard & Ramm, 1988b)*

Cage-side observations for overt signs of toxicity were made.(twice daily). The weights of the animals were recorded (at the start of the study and weekly thereafter). Weekly food consumption and water intake were determined. Clinical laboratory examinations of all the mice were carried out at end of study. After eight weeks all the survivors were sacrificed and grossly appraised. The following organs were weighed: heart, testicles, ovaries, liver, lung, spleen, kidneys, and adrenals. The following tissues were subject to histopathological examination: aorta, eye, caecum, duodenum, jejunum, ileum, colon, femur, brain, Harder's glands, urinary bladder,

skin, heart, testicles, pituitary, bone marrow, liver, lung, lymph node, stomach, spleen, muscle, epididymis, adrenals, nervous ischiadicus, kidneys, oesophagus, ovaries, pancreas, prostate, rectum, seminal vesicle, thyroid, salivary gland, sternum, thymus, trachea, uterus, tongue.

In a satellite study for enzyme induction in the liver (N-demethylase, O-demethylase, P-450) and on triglycerides content, groups of five male and female NMRI mice were administered tebuconazole at doses of 0, 125, 500 and 2000 ppm in the diet for five days (which were equivalent to approximate doses of 0, 31, 125 and 500 mg/kg bw/day).

Results

Clinical observations

No treatment-related deaths or effects on the appearance and behaviour of the mice were observed. Some animals across all groups died, most likely as a consequence of the blood sampling procedure.

Body weight and food intake

Treated animals (both sexes and dose groups) showed a decrease in body weight during the study; consequently a bodyweight loss was recorded for treated animals (both sexes and dose groups), compared to a small bodyweight gain in controls. Bodyweights recorded were < 10 % change compared to control during the majority of the study duration, consequently it was not considered toxicologically relevant. The treated male and female mice consumed about the same amount of feed as the controls.

Table 6.3-16. Body weights (g) (and % difference to control)

	Tebuconazole (ppm)				
	0	**500**	***(%)[a]***	**2000**	***(%)[a]***
Males					
Week 0	39.1	44*	*(+13)*	43.2	*(+10)*
Week 1	29	41.4	*(+43)*	39.8	*(+37)*
Week 2	38.6	40.9	*(+6)*	39	*(+1)*
Week 3	39.2	40.6	*(+4)*	38.5	*(-2)*
Week 4	38.9	39.8	*(+2)*	37.9	*(-3)*
Week 5	38.5	39.5	*(+3)*	37.3	*(-3)*
Week 6	38.4	39.9	*(+4)*	37.7	*(-2)*
Week 7	40.5	41.4	*(+2)*	39.4	*(-3)*
Week 8	39.7	40.1	*(+1)*	37.6	*(-5)*
Bodyweight gain week 0-8	0.6	-3.9		-5.6	
Females					
Week 0	28.9	30.2	*(+4)*	29.4	*(+2)*
Week 1	28.1	29.1	*(+4)*	28.5	*(+1)*
Week 2	28.2	28.7	*(+2)*	26.8	*(-5)*
Week 3	28.3	29.4	*(+4)*	27.6	*(-2)*
Week 4	28.3	29.6	*(+5)*	27	*(-5)*
Week 5	28.6	29	*(+1)*	26.4	*(-8)*
Week 6	28.8	29.5	*(+2)*	27	*(-6)*
Week 7	31.3	30.5	*(-3)*	28.5	*(-9)*
Week 8	30.2	29.5	*(-2)*	28.4	*(-6)*
Bodyweight gain week 0-8	1.3	-0.7		-1	

* *p < 0.05* ** *p < 0.01*

(%)[a] percent change compared to control

Table 6.3-17. Mean daily food intake

	Tebuconazole (ppm)		
	0	**500**	**2000**
Males			

	Tebuconazole (ppm)		
	0	**500**	**2000**
g/animal	620	623	642
g/animal – per day	13	13	13
Females			
g/animal	634	662	645
g/animal – per day	13	14	13

Clinical chemistry

Serum iron was increased in both sexes and treatment groups, which was statistically significant in males at 2000 ppm (+ 25.8 % change compared to control) (Table 6.3-18.). In males indirect bilirubin was statistically significantly reduced at 500 ppm and 2000 ppm in males (> 10 % change compared to control); however, as this was not associated with a reduction of direct bilirubin, it was not considered toxicologically relevant. Overall, serum iron levels were increased from 2000 ppm.

Table 6.3-18. Clinical chemistry data of 8-week toxicity study in mice

Dose [ppm]	**Males**			**Females**		
	0	**500**	**2000**	**0**	**500**	**2000**
Bilirubin total [µmol/L]	3.8	2.8	2.5*	3.3	2.8	3.2
(%)[a]	-	*(-26.3)*	*(-34.2)*	-	*(-15.2)*	*(-3.0)*
Bilirubin direct [µmol/L]	1.2	1.8	1.4	1.2	1.1	0.4
(%)[a]	-	*(+50.0)*	*(+16.7)*	-	*(-8.3)*	*(-66.7)*
Bilirubin indirect [µmol/L]	2.6	1.0*	1.1*	2.2	1.8	2.8
(%)[a]	-	*(-61.5)*	*(-57.7)*	-	*(-18.2)*	*(+27.3)*
Iron [µmol/L]	41.1	44.3	51.7**	47.4	51.9	54.3
(%)[a]	-	*(+7.8)*	*(+25.8)*	-	*(+9.5)*	*(+14.6)*

* statistically significant difference from control p≤0.05
** statistically significant difference from control p≤0.01
(%)[a] percent change compared to control

In the satellite study for liver enzyme induction, in females from 125 ppm and above all four parameters (N-demethylase, O-demethylase, Cyt P-450 and triglycerides) were increased (> 10 % change compared to control), with statistical significance achieved for increases of N-demethylase activity and cytochrome P-450 content (Table 6.3-19). In males the N-demethylase activities were unaffected, whereas the O-demethylase activity was statistically significantly increased at 2000 ppm (> 10 % change compared to control). Cytochrome P-450 and triglyceride content however were statistically significantly increased in males at all doses (> 10 % change compared to control). Overall, liver enzyme induction and triglyceride content were increased from 125 ppm.

Table 6.3-19. Liver enzyme induction data of 5-day toxicity study in mice

Dose [ppm]	**Males**				**Females**			
	0	**125**	**500**	**2000**	**0**	**125**	**500**	**2000**
N-demethylase [mU/g]	234.5	225.2	288.9	222.1	217.2	490.7**	556.6**	364.2**
(%)[a]	-	*(-4.0)*	*(+23.2)*	*(-5.3)*	-	*(+125.9)*	*(+156.3)*	*(+67.7)*
O-demethylase [mU/g]	47.9	48.4	54.6	86.1**	48.9	64.4	77.6**	92.4**
(%)[a]	-	*(+1.0)*	*(+14.0)*	*(+79.7)*	-	*(+31.7)*	*(+58.7)*	*(+89.0)*
Cyt. P-450 [nmol/g]	36.7	55.8**	107.0**	131.9**	32.1	45.6*	94.6**	110.5**
(%)[a]	-	*(+52.0)*	*(+191.6)*	*(+259.4)*	-	*(+42.1)*	*(+194.7)*	*(+244.2)*
Triglycerides [µmol/g]	4.29	12.71**	18.84**	23.78**	5.29	10.84	23.45**	31.76**
(%)[a]	-	*(+196.3)*	*(+339.2)*	*(+454.3)*	-	*(+104.9)*	*(+343.3)*	*(+500.4)*

* statistically significant difference from control p≤0.05
** statistically significant difference from control p≤0.01
(%)[a] percent change compared to control

Gross pathology and histopathology

Pale, slightly swollen livers were seen at necropsy in most treated males, with increased lobulation (Table 6.3-20). The females in the 2000 ppm group also exhibited pale livers. In both sexes, the absolute and relative liver weights were increased (> 15 % change compared to control) from 500 ppm (Table 6.3-21). As this finding was seen in both sexes and occurred in a dose dependent manner, this finding is considered to be adverse and treatment-related. In the males the relative heart weights are also increased, but a dose correlation and thus treatment-relationship is not apparent. Overall, increased liver weights with associated lobulation and pale aspect were seen from 500 ppm.

Table 6.3-20. Gross pathology of liver

	Males			Females		
Dose [ppm]	**0**	**500**	**2000**	**0**	**500**	**2000**
Liver						
Number of animals examined	5	4	5	4	5	5
pale	0	1	1	0	1	4
enlarged / swollen	0	0	3	0	0	0
bile duct proliferation	0	0	3	0	0	0
Kupffer cells	0	2	1	0	2	1
cell degeneration	0	4	5	0	5	5
single cell necrosis, focal	0	0	2	0	0	2
necrotic focus, focal	0	0	1	0	0	0
large vacuoles	0	1	1	0	3	3
Fat content (Oro stain score)	4/5 (1)	4/4 (3)	5/5 (3.2)	4/4 (2)	5/5 (3.4)	5/5 (3.8)

Table 6.3-21. Selected organ weight data of 8-week toxicity study in mice

Dose [ppm]	**Males**			**Females**		
	0	**500**	**2000**	**0**	**500**	**2000**
Liver, absolute [mg]	1995	2661*	2846*	1555	2125*	2090*
(%)[a]	-	*(+33.4)*	*(+42.7)*	-	*(+36.7)*	*(+34.4)*
Liver, relative [mg/100 g]	4802	6435*	7204**	4971	6960*	7394*
(%)[a]	-	*(+34.0)*	*(+50.0)*	-	*(+40.0)*	*(+48.7)*
Heart, absolute [mg]	199	248	234	169	165	157
(%)[a]	-	*(+24.6)*	*(+17.6)*	-	*(-2.4)*	*(-7.1)*
Heart, relative [mg/100 g]	488	601*	593*	540	538	551
(%)[a]	-	*(+23.2)*	*(+21.5)*	-	*(-0.4)*	*(+2.0)*

* statistically significant difference from control $p \leq 0.05$

** statistically significant difference from control $p \leq 0.01$

(%)[a] percent change relative to control

All animals in the 2000 ppm group had increased fat content and liver cell degeneration; in some cases also individual necrosis of hepatocytes and vacuoles were observed (Table 6.3-22). Comparison of the macroscopic findings with the histopathological results shows that all animals with macroscopic liver alterations also had liver cell degeneration, histopathologically. An increased level of ferriferous pigment in the spleen was found in all animals at 2000 ppm. The adrenal cortex cells of all the 2000 ppm males had an increased lipid level (no information provided on females). These findings, except for the liver cell necrosis and spleen pigment, were also found in the 500 ppm group, and are regarded as treatment-related. Based on this, a no-effect level could therefore not be established. Overall, histopathological effects on the liver (degeneration) and adrenals (increased lipids) were seen from 500 ppm. In addition, effects on the spleen (pigment deposition) were seen at the top dose of 2000 ppm.

Table 6.3-22. Incidences of histopathological findings in the 8-week toxicity study in mice

Dose [ppm]	Males 0	Males 500	Males 2000	Females 0	Females 500	Females 2000
Liver						
Number of animals examined	5	4	5	4	5	5
Liver cell degeneration						
Slight	0	0	0	0	1	0
Slight to Moderate	0	0	1	0	3	2
Moderate	0	4	4	0	1	3
Severe	0	0	0	0	0	0
Total	0/5	4/4	5/5	0/4	5/5	5/5
Individual necrosis						
Slight	0	0	1	0	0	1
Slight to Moderate	0	0	1	0	0	1
Moderate	0	0	0	0	0	0
Moderate to Severe	0	0	0	0	0	0
Severe	0	0	0	0	0	0
Total	0/5	0/4	2/5	0/4	0/5	2/5
Large vacuoles						
Slight	0	1	1	0	1	3
Slight to Moderate	0	0	0	0	1	0
Moderate	0	0	0	0	1	0
Moderate to Severe	0	0	0	0	0	0
Severe	0	0	0	0	0	0
Total	0/5	1/4	1/5	0/4	3/5	3/5
Fat content (Oro stain score)						
Slight	4	0	0	1	0	0
Slight to Moderate	0	0	1	2	0	0
Moderate	0	4	2	1	4	1
Moderate to Severe	0	0	2	0	1	4
Severe	0	0	0	0	0	0
Total	4/5	4/4	5/5	4/4	5/5	5/5
Spleen						
Number of animals examined	5	./.	5	3	./.	5
Pigment increased						
Slight	5	./.	0	3	./.	0
Slight to Moderate	0	./.	5	0	./.	5
Moderate	0	./.	0	0	./.	0
Moderate to Severe	0	./.	0	0	./.	0
Severe	0	./.	0	0	./.	0
Total	5/5	./.	5/5	3/3	./.	5/5
Adrenals						
Number of animals examined	5	4	5	./.	./.	./.
Cortex cells lipid-rich						
Slight	0	3	0	./.	./.	./.
Slight to Moderate	0	1	0	./.	./.	./.
Moderate	0	0	5	./.	./.	./.
Moderate to Severe	0	0	0	./.	./.	./.
Severe	0	0	0	./.	./.	./.
Total	0/5	4/4	5/5	./.	./.	./.

Conclusion
In this limited range-finding 28-day dietary study in mice appearance, behaviour and mortality were unaffected by the treatment. Food and water intake were not affected by the treatment.

Serum iron levels were increased from the lowest dose of 500 ppm (equivalent to 82 and 114 mg/kg bw/day in males and females respectively). Increased liver weights (relative and absolute) with associated lobulation and

pale aspect were seen from 500 ppm in both males and females. In both sexes an increase in relative and absolute liver weights was noted from 500 ppm.

Histopathological effect on the liver (degeneration) was seen from 500 ppm and above. In addition, effects on the spleen (pigment deposition) were seen at the top dose of 2000 ppm (equivalent to 329 and 454 mg/kg bw/day in males and females respectively) in both sexes. In 500 and 2000 ppm males, adrenal cortex cells showed an increased lipid content. In the satellite study induction of microsomal enzyme systems was seen in the liver from 125 ppm (equivalent to an approximate dose of 31 mg/kg bw/day) in males and females.

Since histopathological and liver-enzyme findings indicated liver toxicity at both doses, a NOAEL was not established. Doses of 0, 20, 60 and 180 ppm were planned for the oncogenicity study in mice (Section B.6.5.1, B.6.5.2/01).

B.6.3.1.3. ***Summary of sub-acute studies***

One 28-day/4-week oral range-finding study in the rat was described in the original DAR (2006) (B.6.3.1.1/01). In addition two new 28-day/4-week oral range-finding studies in the mouse were submitted for the purpose of renewal (B.6.3.1.2/01 and B.6.3.1.2/02).

Rat
In a 28-day dietary study in rats, a NOAEL of 30 mg/kg bw/d was identified based on effects on some haematological parameters, changes in the weight of liver and spleen and sideropenia in females from 100 mg/kg bw/day. Additional effects were seen in bodyweight development (reduced), some clinical-chemistry parameters (mainly indicative of liver damage), liver pathology and behaviour (lethargy) at the highest dose of 300 mg/kg bw/day. However, some effects were reversible (body weight, haematology, clinical chemistry and enzyme activity).

Mouse
In two 28-day gavage range-finding studies in mice, no NOAEL was set. Treatment-related effects on liver (increase in weights together with histopathological effects) and increased lipid accumulation in the hepatocytes were evident at a LOAEL of 13 mg/kg bw/day. Additional effects were seen in bodyweight development (reduced), clinical-chemistry parameters (increased transaminases, alkaline phosphatase activities and serum ion levels, reduced cholesterol), spleen (pigment deposition) and in the lipid content of adrenal cortex (increase) at higher doses. Marked liver toxicity (including fatty degeneration/vacuolation and increases in liver weights) was also evident in long-term toxicity studies (B.6.5.2/01, B.6.5.2/02).

B.6.3.2. Sub-chronic oral studies (90-day)

Two 90-day/13-week oral studies were described in the original DAR (2006), one in the rat (B.6.3.2.1/01) and one in the dog (B.6.3.2.2/01). These studies were conducted in 1984, prior to latest revisions of the relevant OECD test guidelines; however deviations to current OECD test guidelines have been discussed and do not affect the overall acceptability of the studies. Short-term toxicity studies (four) ranging from 3 to 6 weeks that used other routes (dermal and inhalation) are discussed separately in section B.6.3.4.

B.6.3.2.1. ***Study in rats***

Previous evaluation	In Tebuconazole DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.3.2.1/01
Study title	Subchronic toxicological study on rats. Feeding study over 13 weeks
Dates	In-life dates: February to May 1984
Test substance	(HWG 1608) Tebuconazole (technical grade)
Purity (%) **Batch no.**	93.4 a.i.+ 4.8 symmetrical isomer (an amount outside specifications given in general) 16007/83
Test animals	Male and female Wistar (BOR:WISW) rats
Groups	10/sex/dose

Dose	0 ppm, 100 ppm (= males: 8.6 mg/kg bw/day, females: 10.8 mg/kg bw/day), 400 ppm (= males: 34.8 mg/kg bw/day, females: 46.5 mg/kg bw/day), and 1600 ppm (= males: 171.7 mg/kg bw/day, females: 235.2 mg/kg bw/day)
Route	In the feed (available ad libitum)
Vehicle	Basal diet, no positive control
GLP	Yes
Guideline	OECD guideline 408 (1981) and "Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals", 1982 (US EPA)
Deviation	In relation to the current OECD guideline 408 (1998) the following deviations were found: - functional observations towards the end of the study were not conducted (not included in the guideline in 1981) - weight of the following organs was not determined : epididymides, uterus, ovaries, thymus, brain - the following tissues were not subject to histopathological examination: spinal cord, parathyroid, female mammary gland, peripheral nerve However, detailed functional observations were performed once during the week prior to initiating the exposure and again during weeks 4, 8 and 13 in the sub-chronic neurotoxicity study (B.6.7.1.2/01) at dose levels of up to 107 and 122 mg/kg bw/d in male and female rats, respectively. In the same study also brain weight and histopathological changes of brain, spinal cord and peripheral nerves were assessed. Histopathological changes of the mammary gland and parathyroid and organ weights of ovaries were assessed in the chronic rat study (B.6.5.1/01at dose levels up to 55 and 86.3 mg/kg bw/d in male and female rats, respectively. Thymus weight was measured in the 28-day immunotoxicity study in rats (B.6.8.2.3/01).
Impact of deviations	Minor – these deviations are not considered to affect the validity of the study.
Acceptable	Acceptable
NOAEL	100 ppm (for males and females), equivalent to 9 and 11 mg/kg bw/day respectively.
Effects at the LOAEL	Minor but statistically significant increase in the cytochrome P-450, growth retardation, and histopathological changes in the adrenal cortex at 400 ppm.

Methods

Groups of 10 male and female Wistar rats each were administered 0, 100, 400 and 1600 ppm tebuconazole in the diet for 13 weeks (doses in mg/kg bw/day given in Table 6.3-23) Animals were inspected at least twice daily and any clinical signs and abnormalities were recorded. Detailed individual inspections were made once weekly; body surfaces, orifices, posture, general behaviour, respiration and excretory products were assessed. After four weeks and before end of study ophthalmological examinations were made of ten males and ten females in control and 1600 ppm dose group. Individual body weights were noted before start of administration and then weekly. Food intake was determined per group from start of study up to and including week 13. Clinical laboratory examinations were made one month after start of study of five males and five females from each dose group. After three months all the surviving animals were anaesthetised and sacrificed; animals were then dissected and grossly appraised.

Table 6.3-23. Study design and doses

Test group		**1**	**2**	**3**	**4**
Concentration in diet	[ppm]	**0**	**100**	**400**	**1600**
Dose per animal [mg/kg bw/day]	Male	0	8.6	34.8	171.7
	Female	0	10.8	46.5	235.2

Results

Clinical observations

Appearance, general behaviour, food and water consumption and mortality rate were unaffected up to and including 400 ppm. At 1600 ppm mortality was increased - 1 for males and 1 for females, compared to 2 for males and 0 for females in controls. There were no variations in appearance, behaviour, liveliness and coat condition up to and including 1600 ppm. Overall, mortality was slightly increased at the top dose (1600 ppm).

Ophthalmoscopic results

No treatment-related damage to the eye was found in ophthalmological and histopathological examinations.

Body weight and food intake

At 1600 ppm food consumption was increased in both sexes. Body weights of males and females at 1600 ppm were statistically significantly lower than controls for much of the study, although not always at a magnitude that indicated adversity (not consistently ≥ 10 % change compared to control). Although body weights were statistically significantly reduced at individual time-points at the mid-dose level of 400 ppm, the changes were minimal (≤ 7% difference from controls) and so not regarded by the RMS as adverse. At the termination of the study body weights were adversely affected (> 10 % change compared to controls) in males of the high-dose group (1600 ppm). (Table 6.3-24). There was no adverse effect on terminal body weight at the low- or mid-dose levels. Overall, body weight development was adversely affected only at 1600 ppm (in males).

Table 6.3-24. Body weight results (g) of 90-day toxicity study

	Males				Females			
Dose [ppm]	**0**	**100**	**400**	**1600**	**0**	**100**	**400**	**1600**
Week 0	82	81	79	75	78	78	74	77
(%)[a]		*(-1)*	*(-4)*	*(-9)*		*(±0)*	*(-5)*	*(-1)*
Week 4	162	167	170	159	141	132	131*	125**
(%)[a]		*(+3)*	*(+5)*	*(-2)*		*(-6)*	*(-7)*	*(-11)*
Week 8	284	277	275	251**	179	170	170	163**
(%)[a]		*(-2)*	*(-3)*	*(-12)*		*(-5)*	*(-5)*	*(-9)*
Week 13	334	317	316*	295**	187	181	180	172*
(%)[a]		*(-5)*	*(-5)*	*(-12)*		*(-3)*	*(-4)*	*(-8)*

[a] % compared to control
* statistically significant difference from control p≤0.05
** statistically significant difference from control p≤0.01

Haematology and clinical chemistry

The haematological examination did not detect any treatment-related adverse effects on the blood in any dose group. The results of the urinalyses and the clinical chemical analyses did not reveal toxicologically significant functional or morphological alterations in the dose range investigated.

Gross pathology and histopathology

The results of gross pathological examination did not reveal toxicologically significant functional or morphological alterations in the dose range investigated. The results of the clinical, gross pathological and histopathological examinations did not reveal any liver damage for males and females up to and including 1600 ppm. At 1600 ppm, however, indications of induction of microsomal enzyme systems (N-demethylase and cytochrome P540, > 10 % change compared to control) were noted in males only, whereas the changes in the other groups and in females were within the variation ranges and thus not regarded as treatment-related. A ~~minor but~~ statistically significant increase (> 10 % change compared to control) in the cytochrome P-450 was noted in the 400 ppm dose group in both sexes as well. However, this was accompanied by a statistically significant (> 15 % change compared to control) decrease in liver weight from 400 ppm in both sexes. Histopathology revealed very slightly increased hemosiderin accumulation in the red spleen pulp in some females in the 1600 ppm dose group (Table 6.3-25.). Histopathologically, increased intra-plasmatic vacuoles in the zona fasciculata of the adrenal cortex were observed in females from 400 ppm and in males in the 1600 ppm dose group; these results are to be regarded as induced by the treatment. Overall, hemosiderin accumulation in the spleen was seen at the top dose with liver enzyme induction and vacuolation of the adrenal cortex observed from 400 ppm.

Table 6.3-25. Results of repeated dose toxicity study

	Males				Females			
Dose [ppm]	**0**	**100**	**400**	**1600**	**0**	**100**	**400**	**1600**
Number of animals	10	10	10	10	10	10	10	10
Mortality	2/10	0/10	0/10	1/10	0/10	0/10	0/10	1/10
Body weight [g]	334	317	316*	295**	187	181	180	172*
Food consumption [g/animal/day]	19	19	19	22	16	16	17	22

Dose [ppm]	Males 0	100	400	1600	Females 0	100	400	1600
Clinical chemistry: liver enzyme activities								
N-demethylase [nmol/g/min]	99.2	108.7	100.2	149.7**	41.9	33.3*	31.6	44.2
(%)[a]	-	*(+9.6)*	*(+1.0)*	*(+50.9)*	-	*(-20.5)*	*(-24.6)*	*(+5.5)*
CYT P450 [nmol/g]	30.3	33.5	38.1**	62.8**	27.8	30.5*	32.0**	33.0**
(%)[a]	-	*(+10.6)*	*(+25.7)*	*(+107.3)*	-	*(+9.7)*	*(+15.1)*	*(+18.7)*
Organ weights								
Liver [mg]	13740	12748	11890**	11553**	7998	7102*	6996**	8185
(%)[a]	-	*(-7.2)*	*(-13.5)*	*(-15.9)*	-	*(-11.2)*	*(-12.5)*	*(+2.3)*
Histopathology								
Spleen: increased siderin content								
Very slight	1	2	1	4	5	2	4	5
Very slight - Slight	0	3	2	1	0	0	1	1
Slight	0	1	0	0	0	1	0	3
Total	1/10	6/10	3/10	5/10	5/10	3/10	5/10	9/10
Adrenals: vacuoles in zona fasciculata								
Very slight	2	1	2	2	0	0	3	1
Very slight - Slight	0	3	2	1	0	0	1	2
Slight	1	0	0	0	0	0	0	4
Slight-Moderate	1	0	0	0	0	0	0	1
Moderate	0	0	1	3	0	0	0	1
Total	4/10	4/10	5/10	6/10	0/10	0/10	4/10	9/10

* statistically significant difference from control $p \leq 0.05$
** statistically significant difference from control $p \leq 0.01$
(%)[a] percent change relative to control

Conclusion
In this 90-day study in rats, tebuconazole was tolerated without adverse effects in male and female rats administered the dietary dose of 100 ppm. Mortality was increased at the top dose of 1600 ppm. Body weight development was affected at 1600 ppm. In addition, hemosiderin accumulation in the spleen was seen at the top dose with liver enzyme induction and vacuolation of the adrenal cortex observed from 400 ppm.

Based on these findings, the LOAEL for males and females was 400 ppm, equivalent to 35 and 47 mg/kg bw/day respectively and the NOAEL for males and females was 100 ppm, equivalent to 9 and 11 mg/kg bw/day respectively. This is the same NOAEL value agreed during the first review of tebuconazole.

B.6.3.2.2. ***Study in dogs***

Previous evaluation	In Tebuconazole DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.3.2.2/01
Study title	HWG 1608 - Subchronic study of toxicity to dogs with oral administration (thirteen weeks feeding study)
Date	In life dates: March to June 1984
Test substance	(HWG 1608) Tebuconazole (technical)
Purity (%)	93.4% a.i.+ 4.8% symmetrical isomer (an amount outside specifications given in general)
Batch no.	16007/83
Test animals	Male and female beagle (bor:beag) dogs
Groups	4/sex/group
Dose	0, 200, 1000 and 5000 ppm
Route	Oral, diet.
Vehicle	Basal diet, no positive control
GLP	Yes

Guideline	OECD guideline 409 (1981) not specified, but in accordance with OECD 409 (1981)
Deviation	In relation to the current OECD guideline 409 (1998) the following deviations were found: - weight of the following organs was not determined: gall bladder, epididymides, uterus, thymus and heart - trachea and spinal cord were not subject to histopathological examination However, heart and thymus weight were determined and trachea and spinal cord were examined histopathologically in the chronic dog studies (B.6.3.3.1/02; B.6.3.3.1/01).
Impact of deviations	Minor – the deviations are minimal, can be compensated by the results of other studies and thus they are not considered to affect the validity of the study.
Historical control data	Added in second amendment to report (2002) Dates: 1984 – 1986 (within 5 years of study conduct) Species: Beagle Dogs Laboratory: Same as study
Acceptable	Acceptable
NOAEL	1000 ppm (for males and females), equivalent to 41 mg/kg bw/day.
Effects at the LOAEL	Lens degeneration (opacity), reduced bodyweight and food consumption, increased thrombocyte counts and marked anisocytosis, hemosiderosis in the liver and spleen, change in organ weights and vacuolation in the adrenal zona fasciculata at the next highest dose of 5000 ppm (equivalent to 212 mg/kg bw/day).

Methods
Tebuconazole was administered to 4 male and 4 female beagle dogs per dose group at dietary concentrations of 0, 200, 1000, and 5000 ppm for 13 weeks, equivalent to 0, 8.5, 41, and 212 mg/kg bw/day (calculated: (substance intake / animal / day) / ((body weight week -1 + week 13) / 2) (Table 6.3-26). The programme of examinations included clinical examinations (reflexes, body temperatures, pulse rates, eyes (ophthalmoscopy), state of health), haematology, clinical chemistry, urinalysis, gross necropsy and histopathology. These were conducted regularly but at various times for different parameters.

Table 6.3-26. Study design and doses

Test group	**1**	**2**	**3**	**4**
Concentration in diet [ppm]	**0**	**200**	**1000**	**5000**
Dose per animal [mg/kg bw/day]	0	8.5	41	212

Results
Clinical observations
One female in the highest dose group was found dead on the second study day after only a single treatment, without having previously shown clinical abnormalities. The cause of the death was probably an acute circulatory collapse. Although a connection with the treatment cannot be completely ruled out this seems to be unlikely as all the other dogs survived during the extent of the study period and were normal in behaviour and appearance.

The reflex tests did not detect pathological findings at any examination time. The examinations of body temperatures and pulse rates did not detect any notable variations between the animals in any of the groups, and thus did not provide any indication of a treatment-related effect.

Ophthalmoscopic results
The ophthalmoscopic examination revealed alterations in the high dose animals (212 mg/kg bw/day) which are attributable to the treatment, due to their relation with dose and their increasing trend during the study. Initially in only a few animals lens opacity was noted, but at the end 4/4 female and 1/4 male animals in the high dose groups were affected (Table 6.3-27).

Table 6.3-27. Incidences of ophthalmoscopic findings

Dose [ppm]	Week	Males				Females			
		0	200	1000	5000	0	200	1000	5000
Lens opacity	-1	-	0/1	0/2	0/1	0/2	0/1	-	0/1
	7	0/1	0/1	0/2	1/2	0/2	0/1	-	0/3
	10	-	-	0/3	1/4	0/3	0/1	-	2/4
	12	0/1	0/1	0/3	1/4	0/3	0/1	-	3/4
	14	-	-	1/3	1/4	-	-	-	4/4
Cornea opacity	-1	-	0/1	1/2	0/1	0/2	0/1	-	0/1
	7	0/1	0/1	1/2	0/1	0/2	0/1	-	0/3
	10	-	-	1/3	0/4	0/3	0/1	-	0/4
	12	1/1	0/1	1/3	0/4	0/3	0/1	-	0/4
	14	-	-	0/3	0/4	-	-	-	0/4
Tapetum lucidum unhomogeneous	-1	-	1/1	0/2	0/1	1/2	0/1	-	0/1
	7	0/1	1/1	1/2	0/2	1/2	0/1	-	0/3
	10	-	-	1/3	0/4	2/3	0/1	-	0/4
	12	0/1	1/1	1/3	0/4	1/3	1/1	-	0/4
	14	-	-	1/3	0/4	-	-	-	0/4
Tapetum lucidum light and/or hypereflecting	-1	-	0/1	0/2	0/1	0/2	1/1	-	1/1
	7	0/1	1/1	1/2	0/2	0/2	1/1	-	1/3
	10	-	-	0/3	0/4	1/3	1/1	-	0/4
	12	0/1	1/1	1/3	0/4	1/3	1/1	-	0/4
	14	-	-	1/3	0/4	-	-	-	0/4
Lens star	-1	-	0/1	1/2	1/1	1/2	0/1	-	0/1
	7	0/1	0/1	1/2	1/2	1/2	0/1	-	1/3
	10	-	-	1/3	3/4	1/3	0/1	-	2/4
	12	0/1	0/1	1/3	4/4	1/3	0/1	-	2/4
	14	-	-	1/3	4/4	-	-	-	2/4
Encrusted secretion around eye	-1	-	0/1	1/2	0/1	0/2	0/1	-	0/1
Immovable white deposit on cornea	7	1/1	0/1	0/2	0/2	0/2	0/1	-	0/3
Mydriasis	7	0/1	0/1	0/2	0/2	1/2	0/1	-	0/3
	12	0/1	0/1	0/3	0/4	1/3	0/1	-	0/4
irregular structures in transparent media	7	0/1	0/1	0/2	1/2	0/2	0/1	-	1/3
	10	-	-	0/3	1/4	0/3	0/1	-	3/4
Fundus slightly unsharp	-1	-	0/1	0/2	0/1	0/2	0/1	-	0/1
	7	0/1	0/1	0/2	0/2	0/2	0/1	-	1/3
	10	-	-	0/3	2/4	0/3	0/1	-	2/4
	12	0/1	0/1	0/3	2/4	0/3	0/1	-	3/4
	14	-	-	0/3	2/4	-	-	-	3/4
Cornea scar covered with small vessels	10	-	-	1/3	0/4	0/3	0/1	-	0/4
	12	0/1	0/1	1/3	0/4	0/3	0/1	-	0/4
	14	-	-	1/3	0/4	-	-	-	0/4

Body weight and food intake

Food intake was only reduced at 5000 ppm (Table 6.3-28.) (although < 10 % change compared to control); water consumption was not affected in any group.

Table 6.3-28. Mean feed consumption

		Tebuconazole (ppm)					
		0	200	*(%)[a]*	1000	*(%)[a]*	5000	*(%)[a]*
Males [kg/animal]	Mean per week, weeks 1-13	2.58	2.58	*(±0)*	2.58	*(±0)*	2.45	*(-5)*

		Tebuconazole (ppm)						
		0	**200**	***(%)[a]***	**1000**	***(%)[a]***	**5000**	***(%)[a]***
Females [kg/animal]	Mean per week, weeks 1-13	2.58	2.57	*(±0)*	2.46	*(-4)*	2.42	*(-6)*

[a] *% compared to control*

Treatment of tebuconazole at 200 ppm and 1000 ppm was tolerated without effect on body weights; a reduction in body weight compared to controls was only seen at the top concentration (5000 ppm) (> 10 % change compared to control, a level regarded as adverse). Overall, treatment-related effects on body weights and reduced food intake were recorded at 5000 ppm.

Table 6.3-29. Body weights before study start and in week 13 (kg)

Dose [ppm]	**Males**				**Females**			
	0	**200**	**1000**	**5000**	**0**	**200**	**1000**	**5000**
Week -1	7.8	7.7	8.0	7.7	7.4	6.8	7.3	7.2
(%)[a]		*(-1)*	*(+3)*	*(-1)*		*(-8)*	*(-1)*	*(-3)*
Week 13	10.3	10.2	9.8	9.2	10.3	9.6	9.7	8.6
(%)[a]		*(-1)*	*(-5)*	*(-10)*		*(-7)*	*(-3)*	*(-17)*

[a]: % compared to control

Haematology and clinical chemistry

At 5000 ppm, thrombocyte counts were increased (> 10 % change compared to control at weeks 7 and 13 for males and all sampling periods for females) and marked anisocytosis was observed (throughout the study period) (Tables 6.3-30 and 6.3-31). Erythrocyte counts, haematocrit and haemoglobin figures were unchanged at all the examination times. Overall, adverse and treatment-related effects on some haematological parameters (increased thrombocyte counts and marked anisocytosis) were observed at the top dose of 5000 ppm.

Table 6.3-30. Haematology

		Tebuconazole (ppm)							
		Males				**Females**			
	Week	**0**	**200**	**1000**	**5000**	**0**	**200**	**1000**	**5000**
ERY (10^{12}/L)	-2	6.337	6.537	6.097	6.780	6.322	6.330	6.870	6.960
	3	6.452	6.975	6.125	7.182	6.600	6.557	7.147	7.160
	(%)[a]	-	*(+8.1)*	*(-5.1)*	*(+11.3)*	-	*(-0.7)*	*(+8.3)*	*(+8.5)*
	7	6.485	6.800	6.162	6.957	6.765	6.510	7.100	7.340
	(%)[a]	-	*(+4.9)*	*(-5.0)*	*(+7.3)*	-	*(-3.8)*	*(+5.0)*	*(+8.5)*
	13	5.952	6.205	5.260	6.092	6.192	6.067	6.707	6.320
	(%)[a]	-	*(+4.3)*	*(-11.6)*	*(+2.4)*	-	*(-2.0)*	*(+8.3)*	*(+2.1)*
HB (g/L)	-2	138.5	147.5	136.0	145.8	139.5	140.0	146.0	156.3
	3	143.5	155.3	134.5	158.3	150.3	145.5	158.0	162.8
	(%)[a]	-	*(+8.2)*	*(-6.3)*	*(+10.3)*	-	*(-3.2)*	*(+5.1)*	*(+8.3)*
	7	*140.5*	*148.0*	*131.5*	*148.5*	*149.5*	*141.3*	*155.5*	*159.8*
	(%)[a]	-	*(+5.3)*	*(-6.4)*	*(+5.7)*	-	*(-5.5)*	*(+4.0)*	*(+6.9)*
	13	*145.0*	*150.5*	*124.3*	*142.3*	*152.8*	*144.5*	*159.5*	*154.5*
	(%)[a]	-	*(+3.8)*	*(-14.3)*	*(-1.9)*	-	*(-5.4)*	*(+4.4)*	*(+1.1)*
HCT (L/L)	-2	0.4262	0.4462	0.4070	0.4555	0.4342	0.4337	0.4562	0.4772
	3	0.4302	0.4737	0.4102	0.4775	0.4542	0.4445	0.4790	0.4877
	(%)[a]	-	*(+10.1)*	*(-4.6)*	*(+11.0)*	-	*(-2.1)*	*(+5.5)*	*(+7.4)*
	7	0.4197	0.4432	0.3915	0.4415	0.4445	0.4180	0.4537	0.4687
	(%)[a]	-	*(+5.6)*	*(-6.7)*	*(+5.2)*	-	*(-6.0)*	*(+2.1)*	*(+5.4)*
	13	0.4027	0.4245	0.3530	0.4075	0.4250	0.4115	0.4490	0.4337
	(%)[a]	-	*(+5.4)*	*(-12.3)*	*(+1.2)*	-	*(-3.2)*	*(+5.6)*	*(+2.0)*
THRO (10^9/L)	-2	325	295.8	363.3	303.5	323	387.8	397.3	293.5
	3	319.5	197	331	328.3	282	314	359	399
	(%)[a]	-	*(-38.3)*	*(+3.6)*	*(+2.8)*	-	*(+11.3)*	*(+27.3)*	*(+41.5)*

		Tebuconazole (ppm)							
		Males				Females			
	Week	0	200	1000	5000	0	200	1000	5000
	7	279	205	306	330	267.5	297	298.5	410.5
	(%)[a]	-	*(-26.5)*	*(+9.7)*	*(+18.3)*	-	*(+11.0)*	*(+11.6)*	*(+53.5)*
	13	283.8	214.8	315	356.5	274.5	277	340.5	398.3
	(%)[a]	-	*(-24.3)*	*(+11.0)*	*(+25.6)*	-	*(+0.9)*	*(+24.0)*	*(+45.1)*

(%)[a] percent change relative to control

Table 6.3-31. Anisocytosis

	Males				Females			
Dose [ppm]	**0**	**200**	**1000**	**5000**	**0**	**200**	**1000**	**5000**
Number of animals examined	4	4	4	4	4	4	4	4
Week -2								
Slight	2	3	2	1	1	2	1	1
Moderate	0	0	0	0	0	0	0	0
Severe	0	0	0	0	0	0	0	0
Total	2/4	3/4	2/4	1/4	1/4	2/4	1/4	1/4
Week 3								
Slight	4	1	3	0	1	2	2	2
Moderate	0	3	1	3	3	2	2	2
Severe	0	0	0	0	0	0	0	0
Total	4/4	4/4	4/4	3/4	4/4	4/4	4/4	4/4
Week 7								
Slight	2	4	4	1	4	4	4	2
Moderate	0	0	0	2	0	0	0	2
Severe	0	0	0	0	0	0	0	0
Total	2/4	4/4	4/4	3/4	4/4	4/4	4/4	4/4
Week 13								
Slight	4	3	3	0	3	3	2	0
Moderate	0	0	1	2	0	0	2	0
Severe	0	0	0	1	0	0	0	4
Total	4/4	3/4	4/4	3/4	3/4	3/4	4/4	4/4

At 1000 ppm the age-induced (physiological) fall in alkaline phosphatase activity was slightly retarded while at 5000 ppm a distinct rise of activity was observed at all sampling time points (> 50 % change compared to control) (Table 6.3-32). The liver N-demethylase activity was slightly (at 200 ppm) or distinctly (at 5000 ppm) increased at the end of the study (from +39 % to +232 % change compared to control at 200 ppm and 5000 ppm respectively, a dose-dependent increase). In addition, the high dose group animals (212 mg/kg bw/day) also exhibited higher cytochrome P-450 concentrations in the liver (+ 86 % change compared to control). At 5000 ppm there was also a decrease of mean albumin content and a simultaneous increase of beta-globulin fraction in serum proteins (> +10 % change compared to control).

Overall, effects on some clinical-chemistry parameters (increased ALP, increased liver N-demethylase, higher cytochrome P-450 in the liver, decreased albumin and increased globulin) were seen at the top dose of 5000 ppm. In addition, increased liver N-demethylase activity was also seen at 1000 ppm. Liver weights (g/kg) were increased at a level which is considered adverse (≥ 15 % change compared to control) only in males at the top dose group (5000 ppm). In addition histopathological findings (of accumulation ferri-ferrous pigments) was only seen in females of the top dose group (5000 ppm). Therefore effects are considered adaptive, rather than adverse and treatment-related.

Table 6.3-32. Clinical chemistry results (both sexes)

		Dose (ppm)			
Parameter	**Week**	**0**	**200**	**1000**	**5000**
ALP [U/L]	-1	235.4	228.6	205.0	216.1

Parameter	Week	Dose (ppm) 0	200	1000	5000
	3	211.6	211.8	199.1	331.1
	(%)[a]	-	*(+0.1)*	*(-5.9)*	*(+56.5)*
	7	185.6	191.3	200.5	476.1
	(%)[a]	-	*(+3.1)*	*(+8.0)*	*(+156.5)*
	13	159.9	172.6	198.6	443.1
	(%)[a]	-	*(+7.9)*	*(+24.2)*	*(+177.1)*
Albumin [%]	-1	56.49	56.40	55.47	57.81
	3	57.67	58.30	57.15	55.90
	(%)[a]	-	*(+1.1)*	*(-0.9)*	*(-3.1)*
	7	57.19	57.01	58.11	54.69
	(%)[a]	-	*(-0.3)*	*(+1.6)*	*(-4.4)*
	13	58.37	58.59	58.91	55.42
	(%)[a]	-	*(+0.4)*	*(+0.9)*	*(-5.1)*
ß-globulin [%]	-1	17.26	17.67	17.84	17.15
	3	17.55	16.99	17.54	18.15
	(%)[a]	-	*(-3.2)*	*(-0.1)*	*(+3.4)*
	7	16.60	16.81	17.21	20.24
	(%)[a]	-	*(+1.3)*	*(+3.7)*	*(+21.9)*
	13	17.29	17.35	17.80	19.55
	(%)[a]	-	*(+0.3)*	*(+2.9)*	*(+13.1)*
CYT. P450 [nmol/g]	13	17.90	15.87	19.54	33.32
	(%)[a]	-	*(-11.3)*	*(+9.2)*	*(+86.1)*
N-demethylase [nmol/ (g x min)]	13	52.45	73.02	96.74	174.25
	(%)[a]	-	*(+39.2)*	*(+84.4)*	*(+232.2)*

ALP: Alkaline Phosphatase
(%)[a] percent change relative to control

Gross pathology and histopathology
Organ weights (most organs analysed – liver, kidney, adrenals, spleen and testes) were increased in males at 5000 ppm; prostate weights were decreased in males at 5000 ppm. In females, decreases in organ weights were evident at 5000 ppm (kidney, adrenals and spleen) and ovary weights increased at 5000 ppm.

In the liver slightly increased accumulation of ferri-ferrous pigments in Kupffer cells was noted in all four females and one male in the 5000 ppm group. Histology revealed slight hemosiderosis in the spleen and liver at 5000 ppm, which indicates an increased level of breakdown of the red blood cells. This increased level of iron pigment accumulation in conjunction with adaptation mechanisms to the increased metabolic rate are considered to be the reason for the mean absolute and relative higher spleen weights in the highest dose group in males. Nevertheless these are marginal effects, since the erythrocyte counts and the haematocrit and haemoglobin figures were unchanged at all the examination times. This points to complete compensation.

In the spleen slightly increased accumulation of ferri-ferous pigments in siderocytes of the red spleen pulp were observed in male animals at 5000 ppm. A very slight heightened vacuole formation in the plasma of cells in the zona fasciculata was noted in one control animal and in one animal in each dose group. An additional female in the 5000 ppm dose group had a heightened vacuole formation in the plasma of cells in the adrenal zona fasciculata (Table 6.3-35). The intensity of the finding and the fact that this was an animal in the high dose group points to a treatment-induced effect. However, this is not a manifestation of cytotoxic damage; the alteration is most likely a non-specific adaptive reaction to the treatment.

Some animals at 5000 ppm showed (very) slight degeneration of the posterior wall of the lens. Cataract-like eosinophile plaques were found; the normal lens structure had broken down in this area. Some animals at 5000 ppm exhibited clear subcapsular cataract lentis in both eyes. The lens capsule was unchanged in all cases. Overall, treatment-related effects in the eye were seen at the top dose of 5000 ppm.

Overall, changes in organ weights were evident at the top dose of 5000 ppm. Histopathologically, hemosiderosis was seen in the liver and spleen at 5000 ppm and slight vacuolation was seen in the adrenal zona fasciculata at 5000 ppm. Treatment-related effects in the eye were seen at the top dose of 5000 ppm.

Table 6.3-33. Absolute and relative organ weights

	Week		Tebuconazole (ppm) 0	200	*(%) [a]*	1000	*(%) [a]*	5000	*(%) [a]*
Males									
Liver	13	(g)	373	360	*(-3)*	402	*(+8)*	403	*(+8)*
		(g/kg)	36	36	*(-2)*	41	*(+13)*	45	*(+23)*
Kidney	13	(mg)	53.3	54.8	*(+3)*	53.3	*(±0)*	57.3	*(+8)*
		(mg/100 g)	5.20	5.40	*(+4)*	5.52	*(+6)*	6.40	*(+23)*
Adrenals	13	(mg)	1.127	1.332	*(+18)*	1.355	*(+20)*	1.475	*(+31)*
		(mg/100 g)	0.110	0.134	*(+22)*	0.141	*(+28)*	0.165	*(+49)*
Spleen	13	(mg)	25.3	31.3	*(+24)*	25.3	*(±0)*	36.5	*(+44)*
		(mg/100 g)	2.45	3.12	*(+27)*	2.55	*(+4)*	4.10	*(+67)*
Testes	13	(mg)	17.10	18.40	*(+8)*	15.75	*(-8)*	17.50	*(+2)*
		(mg/100 g)	1.67	1.83	*(+9)*	1.62	*(-3)*	1.96	*(+17)*
Prostate	13	(mg)	5.13	3.66	*(-29)*	3.46	*(-32)*	2.62	*(-49)*
		(mg/100 g)	0.50	0.36	*(-29)*	0.37	*(-25)*	0.29	*(-42)*
Females									
Liver	13	(mg)	377	332	*(-1)*	364	*(-3)*	339	*(-10)*
		(mg/100 g)	37	35	*(+1)*	37	*(-2)*	40	*(+9)*
Kidney	13	(mg)	53.0	45.5	*(-12)*	50.8	*(-6)*	56.5	*(-38)*
		(mg/100 g)	5.20	4.75	*(-10)*	5.22	*(-6)*	6.67	*(-25)*
Adrenals	13	(mg)	1.097	1.027	*(-4)*	1.105	*(-2)*	1.150	*(-32)*
		(mg/100 g)	0.108	0.107	*(-2)*	0.114	*(-1)*	0.137	*(-17)*
Spleen	13	(mg)	33.5	34.0	*(+1)*	29.3	*(-6)*	40.0	*(-31)*
		(mg/100 g)	3.32	3.55	*(+3)*	3.10	*(-5)*	4.75	*(-16)*
Ovary	13	(mg)	1.057	1.025	*(-3)*	0.705	*(-33)*	1.460	*(+38)*
		(mg/100 g)	0.104	0.107	*(+3)*	0.074	*(-29)*	0.173	*(+67)*

[a] *% difference compared to control*
statistical analysis was not performed

Table 6.3-34. Histopathological findings

Dose [ppm]	Males 0	200	1000	5000	Females 0	200	1000	5000
Number of animals	4	4	4	4	4	4	4	4
Liver								
Accumulation ferri-ferrous pigments								
Very slight	1	1	0	2	0	0	0	1
Slight	0	0	0	0	0	0	0	3
Moderate	0	0	0	0	0	0	0	0
Severe	0	0	0	0	0	0	0	0
Total	1/4	1/4	0/4	2/4	0/4	0/4	0/4	4/4
Increased congestion								
Very slight	0	0	0	0	0	0	0	0
Slight	1	0	0	0	0	0	0	1
Moderate	0	0	0	0	0	0	0	0
Severe	0	0	0	0	0	0	0	0
Total	1/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4
Light cells								
Very slight	0	0	0	1	0	0	0	0
Slight	0	0	0	0	0	0	0	0
Moderate	0	0	0	0	0	0	0	0
Severe	0	0	0	0	0	0	0	0
Total	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4

Dose [ppm]	Males				Females			
	0	**200**	**1000**	**5000**	**0**	**200**	**1000**	**5000**
Plasma lightening in hepatocytes								
Very slight	0	0	0	2	0	0	0	0
Slight	0	0	0	0	0	0	0	0
Moderate	0	0	0	0	0	0	0	0
Severe	0	0	0	0	0	0	0	0
Total	0/4	0/4	0/4	2/4	0/4	0/4	0/4	0/4
In hepatocytes cellular infiltration								
Very slight	0	0	0	1	0	0	0	0
Slight	0	0	0	0	0	0	0	0
Moderate	0	0	0	0	0	0	0	0
Severe	0	0	0	0	0	0	0	0
Total	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4
Spleen								
Accumulation ferri-ferrous pigments								
Very slight	0	0	0	2	0	0	1	0
Slight	0	1	0	0	1	1	0	1
Moderate	0	0	0	0	0	0	0	0
Severe	0	0	0	0	0	0	0	0
Total	0/4	1/4	0/4	2/4	1/4	1/4	1/4	1/4
Capsule Thickening								
Very slight	0	0	0	0	0	0	0	0
Slight	0	0	0	0	0	0	0	1
Moderate	0	0	0	0	0	0	0	0
Severe	0	0	0	0	0	0	0	0
Total	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4
Increased congestion								
Very slight	0	0	0	0	0	0	0	0
Slight	0	1	0	0	0	1	0	0
Moderate	0	0	0	0	0	0	0	0
Severe	0	0	0	0	0	0	0	0
Total	0/4	1/4	0/4	0/4	0/4	1/4	0/4	0/4
Adrenals								
Vacuoles in cells of zona fasciculata								
Very slight	0	0	0	0	0	0	1	1
Slight	0	0	0	0	1	1	0	0
Moderate	0	0	0	0	0	0	0	1
Severe	0	0	0	0	0	0	0	0
Total	0/4	0/4	0/4	0/4	1/4	1/4	1/4	2/4
Eyes								
Degenerative alteration in posterior wall of lens								
Very slight	0	0	0	3	0	0	0	1
Slight	0	0	0	1	0	0	0	0
Moderate	0	0	0	0	0	0	0	0
Severe	0	0	0	0	0	0	0	0
Total	0/4	0/4	0/4	4/4	0/4	0/4	0/4	1/4
Cataract lentis								
Very slight	0	0	0	0	0	0	0	0
Slight	0	0	0	0	0	0	0	0
Moderate	0	0	0	0	0	0	0	2
Severe	0	0	0	0	0	0	0	0
Total	0/4	0/4	0/4	0/4	0/4	0/4	0/4	2/4
Brain								
Malignant astrocytoma	0	0	0	0	1	0	0	0

Note: Ferri-ferous pigments (in the liver and spleen) is the basis for hemosiderosis in liver and spleen mentioned in the

Dose [ppm]	Males				Females			
	0	200	1000	5000	0	200	1000	5000

text.

Table 6.3-35. Adrenal findings

Dose [ppm]	Males				Females			
	0	200	1000	5000	0	200	1000	5000
Number of animals	4	4	4	4	4	4	4	4
Cortical nodule	0	0	0	2	2	0	0	0
Adrenals - histopathology: vacuoles in cells of zona fasciculata	0	0	0	0	1	1	1	2

Conclusion
In this 90-day study in dogs, tebuconazole was tolerated without adverse effects at the dietary doses of 200 ppm (equivalent to 8.5 mg/kg bw/day) during a 13 week period.

The only effect recorded in animals dosed at 1000 ppm (equivalent to 41 mg/kg bw/day) was an increase in liver N-demethylase activity. This was not accompanied by changed in liver weight or histopathological findings and is therefore indicative of enzyme induction, an adaptive response. Consequently, the RMS concludes that no adverse effects were induced at this dose.

The effects recorded in the high-dose group (5000 ppm, equivalent to 212 mg/kg bw/day) comprised progressively decreased food consumption and lens degeneration (opacity), indications of anaemia (slightly increased thrombocyte counts and marked anisocytosis, slight siderosis in the spleen, with parallel increases in spleen weight and liver), and in a single female moderate increase in vacuole formation in the cortex of the adrenals.

Based on these effects the LOAEL was therefore 5000 ppm (equivalent to 212 mg/kg bw/day) and the NOAEL 1000 ppm (equivalent to 41 mg/kg bw/day). Due to the conclusion that enzyme induction was an adaptive rather than adverse response, this is a different NOAEL value compared to that agreed during the first review of tebuconazole.

B.6.3.2.3. *Summary of sub-chronic studies*

Two 90-day/13-week oral studies were described in the original DAR (2006), one in the rat (B.6.3.2.1/01) and one in the dog (B.6.3.2.2/01). These studies were conducted in 1984, prior to latest revisions of the relevant OECD test guidelines, however deviations to current OECD test guidelines have been discussed and do not affect the overall acceptability of the studies.

Rat
In the rat, minor but statistically significant increase in cytochrome P-450, body weight development and histopathological changes in the adrenal cortex were observed from 400 ppm. Mortality was increased at the top dose of 1600 ppm. Consequently the LOAEL was 400 ppm, equivalent to 35 and 47 mg/kg bw/day for males and females respectively and the NOAEL was 100 ppm, equivalent to 9 and 11 mg/kg bw/day for males and females respectively. Findings in this 90-day study were similar to those of the two year study in rats.

Dog
In the dog, an increase in liver N-demethylase and a reduction in body weight gain were observed at 1000 ppm. A range of treatment-related effects were observed at the top dose of 5000 ppm; these included a reduction in food intake, ophthalmoscopic alterations, effects on some haematological and clinical-chemistry parameters, changes in organ weights and histopathological effects in the liver, spleen and adrenals. Consequently the LOAEL was 5000 ppm (equivalent to 212 mg/kg bw/day) and the NOAEL was 1000 ppm (equivalent to 41 mg/kg bw/day). A two year study on dogs was not conducted.

Reductions in body weight gain were seen in both the rat and dog. While the overall NOAELs and LOAELs were similar for the two species, a greater range of effects were evident in the dog.

B.6.3.3. Chronic oral studies (12-month)

Two 12-month oral studies in the dog were described in the original DAR (2006) (B.6.3.3.1/01and B.6.3.3.1/02). These studies were conducted in the 1980s, prior to latest revisions of the relevant OECD test guidelines, however deviations to current OECD test guidelines have been discussed and do not affect the overall acceptability of the studies.

B.6.3.3.1. *Studies in dogs*

Two studies are available.

a)

Previous evaluation	In Tebuconazole DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.3.3.1/01
Study title	HWG 1608 – Study of chronic toxicity to dogs after oral administration (twelve month feeding study)
Dates	In life dates: Day of first treatment 20.08.1984; autopsies on 19/20.08.1985.
Test substance	(HWG 1608) Tebuconazole (technical)
Purity (%) **Batch no.**	96.9 Fl. 132 (mixed batch made up of batches 16001/84, 16002/84, 16003/84, 16004/84, 16006/84)
Test animals	Male and female (1:1) beagle (bor:beag) dogs 24 to 28 weeks of age and 7.1 to 10.5 kg of weight
Groups	4/sex/group
Dose	Control: 0 ppm; low dose: 40 ppm; medium dose: 200 ppm; high dose: 1000 ppm (from 1st to 39th week) and 2000 ppm (from 40th to 52nd week)
Route	Dietary administration
Vehicle	Basal diet, no positive control
GLP	Yes
Guideline	In accordance with OECD 452 (1981), though not stated in the study report Note that the current guideline was adopted in 2009
Deviation	In relation to the current OECD guideline 452 (2009) the following deviations were found: - the high dose was changed from 1000 ppm to 2000 ppm during the study (at week 39) in order to test toleration of higher substance concentrations and to produce a clear toxic effect - weight of the following organs was not determined: epididymides and uterus - cervix, coagulating gland, Harderian gland, lacrimal gland, seminal vesicle, spinal cord, trachea and vagina were not subject to histopathological examination However, some of the missing parameters were assessed within the second chronic dog study (B.6.3.3.1/02).
Impact of deviations	Minor – the deviations are minimal, can be compensated by the results of other studies and thus they are not considered to affect the validity of the study.
Acceptable	Acceptable
NOAEL	Males and females: 40 ppm, equivalent to 1.6 mg/kg bw/day
Effects at the LOAEL	Lens alterations (opacity), increased incidence of livers with lobulation, intra-cytoplasmic vacuoles in cells of zona fasciculata of adrenals (females) at 200 ppm, equivalent to 8 mg/kg bw/day for males and females.

Methods
Groups of four male and four female beagle dogs were treated with three dose levels of test substance by oral feed at 0, 40, 200 and 1000/2000 ppm. During the study, the high dose was increased from 1000 ppm to 2000 ppm in order to test tolerability of higher substance concentrations and to produce a clear toxic effect. All the animals were given the same quantity of food in the morning. The food not consumed until the next feeding time was weighed, so that the amount of food consumed, and consequently the amount of test substance administered, were

individually determined.

Regular analyses throughout the study ensured that the food-substance mixes actually contained the specified concentrations of tebuconazole. Before start of study it was established that the test substance was stable for at least fourteen days in the dry feed, and at least 24 hours in the wet feed, and was homogeneously distributed in the mixture.

The laboratory examinations covered clinical findings, food/water intake, haematology, clinical chemistry and urinalyses, gross and histopathology. Along with the continuous inspections of the animals state of health, the examination program included the taking of temperature and pulse rates, reflex tests and ophthalmoscopic examinations (at the time periods shown in the below tables).

Uptake of test substance was 40 ppm: 5.6 g/animal total and 108.4 mg/animal per week; 200 ppm: 28.1 g/animal total and 541 mg/animal per week and 1000 ppm/2000 ppm: 175.8 g/animal total and 3380.2 mg/animal per week.

Table 6.3-36. Study design and doses

Test group	**1**	**2**	**3**	**4**
Concentration in diet [ppm]	0	40	200	1000/2000
Uptake [g/animal]	0	5.6	28.1	175.8
Uptake [mg/animal/week]	0	108.4	541	3380.2
Dose per animal [mg/kg bw/day]	0	1.6	8	47.3

Results

***Clinical sign*s**

No mortalities at any dose were observed. The animals in all groups did not differ from each other in appearance and behaviour. Common findings such as vomiting, pasty faeces and diarrhoea were found in controls and all dose groups with no dose correlation. This however, did not affect body weight and food consumption of the animals as these parameters were unaffected.

No treatment-related effects were seen on reflexes, body temperature or pulse rates.

Body weight and food intake

A treatment-related reduction in food consumption was not noted. The animals consistently finished their food, with a few exceptions without dose correlation. Only some female dogs in all the groups left food more frequently. It should however be noted here that the daily food ration was based on all the animals' mean body weight, and consequently the lighter (female dogs) were over-fed. The dogs in all groups gained weight during the twelve months of the study; the mean gains were between + 2.85 kg to + 5.5 kg.

Ophthalmoscopic examination

The 40 ppm animals tolerated the twelve months of treatment without toxicologically-relevant changes in the transparent media (cornea, anterior chamber of eye, lens, vitreous body) and in the fundus of the eye. One female dog at 200 ppm and at the highest dose (both from the 26th week) exhibited alterations in the lens (lens opacity) (Table 6.3-37). These were central stellar lens opacities or lens stars which were not apparent at the preliminary examination (week -2). The findings were noted in the dogs at 200 ppm for the first time in the 26th week (lens opacity) and 32nd week (lens star), and from then on were apparent at the same intensity at all the following examination times. In the dog treated with the highest dose, a fine stellar lens opacity was seen for the first time in the 26th week and then also in the 32nd week but not from the 39th to 52nd weeks. All other animals in this group had no lens stars, or the lens star had already been faintly present at the preliminary examination and did not become more pronounced under the treatment. Overall, eye lesions were seen from 200 ppm.

Table 6.3-37. Ophthalmoscopic results

Dose [ppm]	**Week**	**Males [N]**				**Females [N]**			
		0	**40**	**200**	**1000/ 2000**	**0**	**40**	**200**	**1000/ 2000**
Number of animals examined		4	4	4	4	4	4	4	4

Dose [ppm]	Week	Males [N]				Females [N]			
		0	40	200	1000/ 2000	0	40	200	1000/ 2000
Lens opacity	-2	0	0	0	0	0	0	1	0
	13	0	0	0	0	0	0	1	0
	26	0	0	0	0	0	0	2	1
	32	0	0	0	0	0	0	2	1
	39	0	0	0	0	0	0	2	0
	46	0	0	0	0	0	0	2	0
	52	0	0	0	0	0	0	2	0
Cornea opacity	-2	0	0	0	0	0	0	0	0
	13	1	1	2	1	0	0	0	0
	26	1	1	2	1	0	0	0	1
	32	1	1	2	1	0	1	0	2
	39	1	0	2	1	0	0	0	2
	46	1	1	1	1	0	0	0	1
	52	1	2	1	1	0	0	0	1
Tapetum lucidum unhomogeneous	-2	0	0	0	1	0	0	0	0
	13	0	0	0	1	0	0	0	0
	26	0	0	0	1	0	0	0	0
	32	0	0	0	1	0	0	0	0
	39	0	0	0	1	0	0	0	0
	46	0	0	0	1	0	0	0	0
	52	0	0	0	1	0	0	0	0
Tapetum lucidum light and/or hypereflecting	-2	0	0	1	0	0	0	1	0
	13	0	0	1	0	0	0	1	0
	26	1	0	1	0	0	1	1	0
	32	1	0	0	0	0	1	1	0
	39	1	0	0	0	0	1	1	0
	46	1	0	0	0	0	1	1	0
	52	1	0	0	0	0	1	1	0
Lens star	-2	0	2	0	1	0	1	1	0
	13	0	2	0	1	0	0	1	0
	26	0	2	0	1	0	0	1	0
	32	0	1	0	1	0	0	2	0
	39	0	1	0	1	0	0	2	0
	46	0	1	0	1	0	0	2	0
	52	0	1	0	0	0	0	2	0
Reflecting Particles	-2	0	1	0	0	0	0	0	0
	13	0	1	0	0	0	0	0	0
	26	0	1	0	0	0	0	0	0
Remnants of lens arteri	32	0	1	0	0	0	0	0	0
	39	0	1	0	0	0	0	0	0
	46	0	1	0	0	0	0	0	0
	52	0	1	0	0	0	0	0	0
Mucous deposits	32	0	1	0	1	0	0	0	0
	39	0	1	0	1	0	0	0	0
	46	0	1	0	1	0	0	0	0
	52	0	1	0	1	0	0	0	0
Vessels convoluted and full	-2	0	0	0	0	0	0	0	1
	13	0	0	0	0	0	0	0	1
	26	0	0	0	0	0	0	0	1
	32	0	0	0	0	0	0	0	1
	39	0	0	0	0	0	0	0	0
	46	0	0	0	0	0	0	0	0

Dose [ppm]	Week	Males [N]				Females [N]			
		0	40	200	1000/ 2000	0	40	200	1000/ 2000
	52	0	0	0	0	0	0	0	0

Haematology and clinical chemistry
No treatment-related changes in haematological or urinary parameters were observed and in general no treatment-related clinical chemistry findings were seen, apart from those related to the liver (described below).

The mean activity of the alkaline phosphatase (AP) showed an age-related decrease in the animals in the control, 40 and 200 ppm groups. Only the high-dose animals (1000/2000 ppm) displayed a slightly retarded decrease in AP activity, between weeks -2 and week 52, compared to the controls. At the top dose (1000/2000 ppm), the decrease in alkaline phosphatase activity was only slight throughout the study and a small increase was evident from the 40th week. However, overall, treatment had a slight effect on alkaline phosphatase activity only at the top dose (1000/2000 ppm).

Liver enzyme determination showed that the N-demethylase activities and triglyceride concentrations were slightly higher in the high-dose group (1000/ 2000 ppm) at the end of the study (+ 95 % and + 38 % compared to control, for N-demethylase and triglyceride respectively) (Table 6.3-39). Overall, there were no treatment-related effects on haematological parameters, but there were a slight increase in AP activity, higher hepatic N-demethylase activity and higher hepatic triglyceride content at the top dose (1000/ 2000 ppm).

The electrophoretic examinations did not reveal any treatment-related changes.

Table 6.3-38. Clinical chemistry results (both sexes)

Parameter	Week	Dose [ppm]			
		0	40	200	1000/2000
AP [U/L]	-2	206.4	206.1	219.9	188.1
	6	150.5	175.6	197.1	171.6
	(%)[a]	-	*(+16.7)*	*(+31.0)*	*(+14.0)*
	13	138.1	150.6	184.6	167.5
	(%)[a]	-	*(+9.1)*	*(+33.7)*	*(+21.3)*
	26	120.0	119.6	163.5	152.5
	(%)[a]	-	*(-0.3)*	*(+36.3)*	*(+27.1)*
	39	87.8	90.1	122.6	142.9
	(%)[a]	-	*(+2.6)*	*(+39.6)*	*(+62.8)*
	46	110.9	-	-	168.4
	(%)[a]	-	-	-	*(+51.8)*
	52	99.8	104.3	138.8	167.4
	(%)[a]	-	*(+4.5)*	*(+39.1)*	*(+67.7)*

AP: Alkaline Phosphatase
(%)[a] percent change relative to control

Table 6.3-39. Liver enzymes (week 52)

Parameter	Dose [ppm]			
	0	40	200	1000/2000
N-demethylase [nmol/g x min]	53.525	39.062	52.600	104.275
(%)[a]	-	*(-27.0)*	*(-1.7)*	*(+94.8)*
CYT P450 [nmol/g]	29.80	23.91	24.12	25.69
(%)[a]	-	*(-19.8)*	*(-19.1)*	*(-13.8)*
Triglyceride [µmol/g]	4.07	4.29	4.44	5.63
(%)[a]	-	*(+5.4)*	*(+9.1)*	*(+38.3)*

(%)[a] percent change relative to control

Gross pathology and histopathology
There was no indication of any treatment-related effect on absolute and relative organ weights for treated animals.

There was a dose-related incidence of livers with increased lobulation noted at autopsy from 200 ppm (2 of 8 dogs in the medium dose group (200 ppm), 5 of 8 dogs in the high dose group (1000/2000 ppm)) (Table 6.3-40). According to the histopathological examination, these alterations were however not the result of morphological apparent liver lesions. Intra-cytoplasmic vacuoles in cells of zona fasciculata of the adrenals were observed in 2 females of the medium dose group (200 ppm) and high dose group (1000/2000 ppm). These findings were regarded as treatment-related, since no similar alterations were found in controls and low dose group (Table 6.3-40). The slightly increased siderin content in the spleens of high-dose dogs (5 of 8 dogs) is considered a substance-induced effect (Table 6.3-40), however this finding had the same intensity as in control dogs. Overall, increased liver lobulation and vacuolation of the zona fasciculata of the adrenals were seen from 200 ppm and increased spleen siderin content was observed at the top dose (1000/2000 ppm).

Table 6.3-40. Pathology findings

Dose [ppm]	**Males [N]**				**Females [N]**			
	0	**40**	**200**	**1000/ 2000**	**0**	**40**	**200**	**1000/ 2000**
Number of animals	4	4	4	4	4	4	4	4
Ophthalmology Lens opacity	0	0	0	0	0	0	2	2
Liver - gross pathology Distinct lobulation	0	0	0	2	0	0	2	3
Adrenals - histopathology: Vacuoles in cells of zona fasciculata	0	0	0	0	0	0	2	2
Spleen: histopathology Siderin content	1	0	1	2	1	3	2	3

Conclusion
In this 1-year dietary study in dogs, tebuconazole was well tolerated at the lowest dose of 40 ppm. At 200 ppm and above there were eye lesions, increased liver lobulation and vacuolation of the zona fasciculata of the adrenals. In addition, at the top dose there were a slight increase in AP activity, higher hepatic N-demethylase activity, higher hepatic triglyceride content and increased spleen siderin content.

Based on these findings a LOAEL of 200 ppm, equivalent to 8 mg/kg bw/day was established for males and females. The NOAEL for males and females was 40 ppm, equivalent to 1.6 mg/kg bw/day. This is the same NOAEL value agreed during the first review of tebuconazole.

b)

Previous evaluation	In Tebuconazole DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.3.3.1/02
Study title	Safety evaluation of HWG 1608: chronic (1 year) feeding study in dogs Addendum: Supplemental submission to EPA MRID No. 42030601: Safety evaluation of HWG 1608: chronic (1 year) feeding study in dogs, Miles Inc.
Dates	In life dates: Start: 30.7.1987, for a minimum of 369 consecutive days
Test substance	(HWG 1608) Tebuconazole (technical)
Purity (%) **Batch no.**	96 16013/86
Test animals	Male and female (1:1) beagle (bor:beag) dogs of 6 month of age and approximately 6-9 kg of weight
Groups	4/sex/group
Dose	Control: 0 ppm, low dose: 100 ppm (= males: 2.69 mg/kg bw; females: 2.94 mg/kg bw), high dose: 150 ppm (= males: 4.39 mg/kg bw; females: 4.45 mg/kg bw)
Route	Dietary administration
Vehicle	Basal diet, no positive control

GLP	Yes
Guideline	FIFRA § 83-1, which correspond to OECD 452 Note that the current guideline was adopted in 2009
Deviation	The study was conducted according to US-EPA FIFRA 83-1, which corresponds to OECD 452. In relation to the current OECD guideline 452 (2009) the following deviations were found: - only two dose levels were used (100 and 150 ppm) - weight of the following organs was not determined: epididymides, ovaries and uterus - caecum, coagulating gland, harderian gland, lacrimal gland, parathyroid gland, seminal vesicle and skin were not subject to histopathological examination However, this study was designed as a targeted supplementary chronic feeding study with HWG 1608 in dogs to establish a higher no-effect level as observed in an earlier chronic study (see B.6.3.3.1/01) and some of the missing parameters were assessed within this earlier study.
Impact of deviations	Minor – the deviations are minimal, can be compensated by the results of other studies and thus they are not considered to affect the validity of the study.
Acceptable	Acceptable as a supplementary chronic toxicity study using the dog as the most sensitive species tested and with the purpose of refining the NOAEL value
NOAEL	100 ppm - equivalent to 3.0 mg/kg bw/day.
Effects at the LOAEL	Hypertrophy of the adrenal zona fasciculata in all animals at 150 ppm (the top dose).

Methods
Groups of four male and four female beagle dogs were treated with two dose levels (100 and 150 ppm) of test substance by oral feed during 12 month. Animals were offered the diets daily for 1 year during which time body weights, food consumption, and clinical pathology parameters were monitored. The animals were observed daily for clinical signs of toxicosis; physical examinations including direct ophthalmoscopy were performed on all dogs pre-treatment, at the end of 3 and 6 months, and at termination; gross and microscopic pathologic examination were performed on all dogs at termination.

Table 6.3-41. Study design and dose

Test group		**1**	**2**	**3**
Concentration in diet	(ppm)	**0**	**100**	**150**
Dose per animal (mg/kg bw/day)	Male	0	2.96	4.39
	Female	0	2.94	4.45

Results
Clinical signs
No mortalities occurred in treated animals and no clinical signs were observed, except sporadic incidences of soft stools/diarrhoea and rare incidences of emesis; these were not regarded as treatment-related. Only one female control animal stopped eating and developed higher temperature. This animal was replaced by another female animal.

Ophthalmoscopic results
No treatment-related effects were seen.

Body weight and food intake
No treatment-related effects on body weight development or feed intake were observed.

Haematology and clinical chemistry
No treatment-related effects on haematological or clinical chemistry parameters were seen.

Pathology and organ weights
There was no indication of any treatment-related effect on gross pathology and absolute and relative organ weights; organ weights of treated animals did not differ significantly from those of controls.

Subtle hypertrophy (minimal to mild) of adrenal zona fasciculata cells occurred in almost all animals in the high-dose group (150 ppm), 4/4 males and 3/4 females, compared to only 1 female control dog (Table 6.3-42). This finding was not accompanied by a change in adrenal weight and appeared to be due to an increase in the size and/or number of lipid vacuoles.

Table 6.3-42. Pathology findings

	Males			Females		
Dose (ppm)	**0**	**100**	**150**	**0**	**100**	**150**
Number of animals examined	4	4	4	4	4	4
Mortality	-	-	-	1/4[1]	-	-
Adrenals:						
Hypertrophy of cells of zona fasciculata						
Minimal	0	0	2	1	0	0
Mild	0	0	2	0	0	3
Moderate	0	0	0	0	0	0
Severe	0	0	0	0	0	0
Total	0/4	0/4	4/4	1/4	0/4	3/4
Lipid hyperplasia						
Minimal	1	1	2	0	1	1
Mild	1	0	0	1	1	1
Moderate	0	0	0	0	0	0
Severe	0	0	0	0	0	0
Total	2/4	1/4	2/4	1/4	2/4	2/4
Fatty change zona glomerulosa						
Minimal	1	1	2	1	1	1
Mild	0	0	1	0	0	1
Moderate	0	0	0	0	0	1
Severe	0	0	0	0	0	0
Total	1/4	1/4	3/4	1/4	1/4	3/4

[1] One female was replaced by another female on day 70 due to anorectic and hypoactive effects

Conclusion
In this limited 1-year dietary study in dogs, the only treatment-related effect was a subtle hypertrophy of the adrenal zona fasiculata in in all animals at 150 ppm (the top dose) which appeared to be due to an increased cell size and/or the number of vacuoles. No adrenal weight changes were seen.

Based on this a LOAEL of 150 ppm, equivalent to 4.4 mg/kg bw/day and a NOAEL of 100 ppm, equivalent to 3.0 mg/kg bw/day was determined. This is the same NOAEL value agreed during the first review of tebuconazole.

B.6.3.3.2. *Summary of chronic studies in dogs*

The chronic oral toxicity of tebuconazole was investigated in the dog in two 1-year studies.

In the first study (B.6.3.3.1/01), treatment-related, adverse effects were seen at the mid-dose of 200 ppm; these consisted of eye lesions and histopathology (increased liver lobulation and vacuolation of the zona fasiculata of the adrenals). In addition, at the top dose (1000/2000 ppm) there were treatment-related effects on clinical chemistry (increase in AP activity, hepatic N-demethylase and hepatic triglyceride content) and increased spleen siderin content. Based on these effects a NOAEL of 40 ppm (1.6 mg/kg bw/day) was therefore identified. In this longer dog study the increase in hepatic N-demethylase and progressive lens degeneration (opacity) seen in the 90-day study (B.6.3.2.2/01) was confirmed.

In the second study (B.6.3.3.1/02), conducted in order to refine the NOAEL, subtle hypertrophy of cells of the zona fasciculata of the adrenals occurred at the top dose of 150 ppm, due to an increase in the size and/or number of lipid vacuoles. Consequently the NOAEL was set at 100 ppm (3 mg/kg bw/day). In this longer dog study the increase in vacuoles in the cells of the zona fasiculata seen in the 90-day study (B.6.3.2.2/01) was confirmed. However, the retarded weight development seen in the 90-day study was not seen in the 1-year studies, probably

due to the lower doses used.

B.6.3.4. Other routes

Short-term toxicity studies by other routes were also available and described in the original DAR (2006): one 3-week dermal study in the rabbit (B.6.3.4/01), one 3-week inhalation study in the rat (B.6.3.4.2/01), and two inhalation studies investigating specifically cataracts, one in the cat (B.6.3.4.3/01) and one in the dog (B.6.3.4.4/01).

B.6.3.4.1. *Sub-acute dermal study in rabbits*

Previous evaluation	In Tebuconazole DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.3.4/01
Study title	HWG 1608 techn. – Sub-acute dermal study of toxicity to rabbits (Addendum to Report no. 12669 of 8.5.1984)
Test substance	(HWG 1608) Tebuconazole (technical)
Purity (%) **Batch no.**	97.4 16012/86
Test animals	Male and female New Zealand White rabbits, strain HC:NZW
Groups	5/sex/dose
Dose	0 and 1000 mg/kg bw (limit test) for 6 h/day – 5 days/week – duration 3 weeks
Route	Dermal – non-occlusive dressing
Vehicle	Cremophor EL 2% in water v/v. The dosage formulation contained 25% w/v active ingredient. Dosage volume 4 mL/kg bw – vehicle control dosage volume 2 mL/kg bw
GLP	Yes
Guideline	OECD guideline 410 (1981) and US EPA guideline § 82-2 (1984)
Deviation	None.
Impact of deviations	N/A
Acceptable	Acceptable
NOAEL	Systemic effects: 1000 mg/kg bw/day (limit test dose with occlusion) Preceding study with 3 rabbits/dose (un-occluded): 250 mg/kg bw/day (the highest dose tested)
Effects at the LOAEL	N/A

Methods
The dermal toxicity study in rabbits was performed in accordance with OECD guideline 410 – limit test. Five rabbits per sex were given dermal applications of 0 or 1000 mg technical tebuconazole/kg bw for 6 hours per day, 5 days per week for 3 consecutive weeks

Results
The dermal treatment with the test substance did not produce any systemic effects on male and female rabbits, which could be attributed to the active ingredient.

The slight alterations in the skin (isolated and temporarily very slight redness) revealed by the clinical and histopathological examinations occurred a little more frequently in males than in females. The alterations are presumably attributable to mechanical irritation of the skin, since the test compound formulation was a suspension of viscous consistency or slurry, and the pressure of the occlusive dressing presumably resulted in skin friction.

In a preceding study by the same authors, but using a different study outline (non-guideline) 3 rabbit/sex/dose/skin treatment (intact or abraded) were administered 0, 50 or 250 mg tebuconazole techn./kg bw suspended in Cremophor EL 2% in water at a dosing volume of 0.5 mL/kg bw for 6 hours/day, 5 days/week for 3 consecutive weeks. The doses were left uncovered so it was not possible to apply a larger volume.

No systemic or local effects were noted during or after the study period with respect to general clinical condition and behaviour of the animals. Body weight (and development), local skin toleration, haematological examinations, clinical chemistry examinations, urinalysis were not affected. No changes were recorded at autopsy, whether microscopical or histopathological in any of the dose groups whether tested in animals with intact or with abraded skin. The NOAEL from this more limited dermal un-occluded study was 250 mg tebuconazole/kg bw/day – the highest dose tested.

Conclusion
In this OECD guideline 410 (limit-)study of the toxicity of dermal application to rabbits of 0, and 1000 mg tebuconazole/kg bw/day under non-occlusive dressing for 6 hours/day, 5 days/week for 3 consecutive weeks the NOAEL for systemic effects was 1000 mg/kg bw/day. Locally minimal alterations of the treated skin were noted, which are most likely attributable to mechanical irritation. This is the same NOAEL value agreed during the first review of tebuconazole.

B.6.3.4.2. ***Sub-acute inhalation study in rats***

Previous evaluation	In Tebuconazole DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.3.4.2/01
Study title	HWG 1608 - Study for subacute inhalation toxicity to the rat for three weeks (exposure 15 x 6 hours)
Dates	In-life dates: March to April 1984
Test substance	(HWG 1608) Tebuconazole
Purity (%) **Batch no.**	96.2 16001/83
Test animals	Wistar rats, strain Bor:WISW III
Groups	10/sex/dose
Dose	0 (vehicle), 1.2, 10.6, or 155.8 mg/m^3 air. Besides a negative control group exposed to air only.
Route	Head-nose exposure to an aerosol of tebuconazole
Vehicle	Ethanol/polyethylene glycol E 400 (= Ethanol/Lutrol) 1:1
GLP	Yes
Guideline	OECD guideline 412 (1981)
Deviation	3 week (21 days) exposure is not mentioned in the guideline (28 days or 14 days).
Impact of deviations	Minor – this deviation is minimal and is not considered to affect the validity of the study.
Acceptable	Acceptable
NOAEL	10.6 mg/m^3 air in both sexes (approx. 3 mg/kg bw/day).
Effects at the LOAEL	Slight clinical signs of toxicity (piloerection) and liver enzyme induction at the next highest concentration (155.8 mg/m^3 air) (the top concentration) (approx. 45 mg/kg bw/day).

Methods
Groups of 10 male and 10 female Wistar rats were head-nose exposed to a tebuconazole aerosol at analytical concentrations of 0, 1.2, 10.6, or 155.8 mg/m^3 air (theoretical concentrations of, 0, 5, 50, or 500 mg/m^3 air) for 3 weeks (15 x 6 hours, 5 times per week).

The mean mass median aerodynamic diameter (MMAD) was between 2.0 – 2.1 µm for all test concentrations, this is within the range stated in the OECD test guideline No. 412 (2009) (1 – 3 µm) and current (2017) guideline (≤ 2 µm). The geometric standard deviation was between 2.0 – 2.1 µm for all test concentrations, this is within the range stated in the current OECD test guideline No. 412 (2017) (1 – 3).

Table 6.3-43. Results of particle analysis MMAD (µm) (and geometric standard deviation)

MMAD (µm)	**Test concentration (mg /m^3 air)**			
	0	**5**	**50**	**500**
BE		1.9 (2.0)		2.5 (2.4)

MMAD (µm)	Test concentration (mg /m³ air)			
	0	5	50	500
BA	2.1 (2.1)	2.3 (2.1)	2.1 (1.9)	2.0 (1.9)
BA	2.0 (2.0)	2.2 (2.0)	2.0 (1.9)	2.1 (2.0)
BA	2.1 (2.1)	2.0 (2.1)	2.0 (2.1)	2.0 (2.0)
BA	1.8 (2.1)	1.9 (1.9)	1.9 (2.0)	2.0 (2.0)
mean	2.0 (2.1)	2.1 (2.0)	2.0 (2.0)	2.1 (2.1)

BE = particle analysis with Berner impactor
BA = particle analysis with Bayer impactor
MMAD = mass median aerodynamic diameter (µm)

During the 3 weeks of exposure body weights, clinical signs and mortality were recorded. At the end of the study clinical chemistry, haematology, urine, gross pathological and histopathological examinations were performed.

Results

Clinical observations

No mortalities due to treatment occurred. The treatment was tolerated without ill-effects by the rats in the groups up to and including 10.6 mg/m³ (Table 6.3-44). The males and females in the 155.8 mg/m³ group showed bristling coats (piloerection) after each exposure. Overall, slight clinical signs of toxicity were seen at the top concentration.

Table 6.3-44. Toxicological results

	Group no				
	1	2	3	4	5
Males					
Concentration (mg/ m³ air) analytical	air	vehicle	1.2	10.6	155.8
Toxicological result	0/0/10	0/0/10	0/0/10	0/0/10	0/10/10
Length sign	--	--	--	--	9d-11d
Time death	--	--	--	--	--
Mortality (%)	0	0	0	0	0
Females					
Concentration (mg/ m³ air) analytical	air	vehicle	1.2	10.6	155.8
Toxicological result	*1/0/10	0/0/10	0/0/10	0/0/10	0/10/10
Length sign	--	--	--	--	7d-9d, 12d - 21d
Time death	11d	--	--	--	--
Mortality (%)	10	0	0	0	0

*= died due to broken neck on insertion into exposure tube (rat no. 19)

Body weight

The body weight development of the animals was not affected.

Haematology, clinical chemistry and urinalysis

The haematological examination did not detect any adverse effects on the blood. The results of the urinalyses and the clinical chemical analyses examination did not reveal toxicologically significant functional or morphological alterations in the concentration range investigated.

The clinical-chemistry examinations made at end of the study revealed a slight plasma GLDH increase in the male rats in the medium and highest groups (Table 6.3-35). This slight increase is not seen as related to treatment, since the values are within the physiological range of variation of untreated control rats (historical controls, 2 standard deviations range of variation for GLDH 0 to 29 U/L), and a clear concentration correlation could not be detected (despite a tenfold concentration difference).

The slight alterations in plasma creatinine concentration are not considered relevant, since the overall changes are slight, and in addition none of the figures exceed the physiological range of variation for untreated rats (historic controls, 2s range of variation/creatinine 24 to 89 µmol/L). The urea results do not show a treatment-related effect

since there were only decreases which in addition were not concentration-related (Table 6.3-45). Overall, there were no treatment-related effects on clinical-chemistry parameters.

Table 6.3-45. Clinical chemistry results

	Dose (mg/m³)				
	0 (Air)	**0 (Solvent)**	**5**	**50**	**500**
Males					
UREA (mmol/L)	9.19	7.80**	7.46**	7.45**	7.37**
CREA (µmol/L)	74	71	60**	52**	51**
GLDH (U/L)	1.1	0.7	1.8	3.7**	3.9**
Females					
UREA (mmol/L)	8.65	7.70**	6.66**	7.27**	7.19**
CREA (µmol/L)	67	65	56*	53**	52**
GLDH (U/L)	1.2	7.4	0.8	2.5	1.8

* p< 0.05, ** p < 0.01

The examinations made at the end of the study revealed a significant increase in N-demethylase activity in the liver tissue of the male and female rats in the highest group (155.8 mg/m³) (Table 6.3-46.). In addition, males in this group exhibited marginal increases in O-demethylase activity. These findings are considered to be related to a slight liver enzyme induction. Overall, therefore, liver enzyme induction was seen at the top concentration of 155.8 mg/m³.

Table 6.3-46. Mixed function oxidases in the liver

	Dose (mg/m³)				
	0 (Air)	**0 (Solvent)**	**5**	**50**	**500**
Males					
N-demethylase (mmol/g/min)	104.2	96.8	113.2	117.4	154.4**
O-demethylase (mmol/g/min)	10.6	9.2	9.4	9.8	12.3*
P-450 (nmol/g)	39.4	39.3	38.9	39.2	42.7
Females					
N-demethylase (mmol/g/min)	44.9	44.0	46.5	46.9	63.9**
O-demethylase (mmol/g/min)	11.1	10.7	11.0	11.0	11.6
P-450 (nmol/g)	24.0	25.3	23.2	23.6	26.3

Urinalysis did not reveal any treatment-related effect.

Sacrifice and pathology
The gross pathological examination of the rats autopsied at end of study did not reveal any indications of grossly apparent organ changes induced by the active ingredient. No toxicologically relevant changes in the absolute organ weights of the animals exposed to tebuconazole in comparison to the control group were noted. The results of the histopathological examination did not reveal toxicologically significant functional or morphological alterations in this study.

Conclusion
In this guideline 3 weeks (6 hours/day and 5 days/week) study, inhalational exposure (head/nose only) of rats to analytical aerosol concentrations of 0, 1.2, 10.6 or 155.8 mg/m³ air and based on slight clinical signs of toxicity (piloerection) and liver enzyme induction at 155.8 mg/m³ (approx. 45 mg/kg bw/day, assuming 100% inhalation absorption) air a NOAEL was established at 10.6 mg/m³ (approx. 3 mg/kg bw/day, assuming 100% inhalation absorption) air in both sexes. This is the same NOAEL value agreed during the first review of tebuconazole.

B.6.3.4.3. *Sub-acute inhalation study in cats - study to investigate cataracts*

Previous evaluation	In Tebuconazole DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.3.4.3/01
Study title	HWG 1608 - (proposed c.n.: tebuconazole) - Subacute inhalation toxicity to cats - study for cataracts
Test substance	(HWG 1608) Tebuconazole
Purity (%) **Batch no.**	95.8 16013/86
Test animals	"Forest of Dean" breed of cats
Groups	4/sex/dose
Dose	0 (negative control), 61, or 309 mg/m^3 air (target concentrations were 0, 50, or 350 mg/m^3 air). Also tested was a positive control KNJ 0953 (99.7% pure – 30 mg/m^3 air)
Route	Whole-body exposure for respirable aerosol (MMAD 1.5-1.6 µm; 99% particles ≤ 5 µm aerodynamic diameter)
Vehicle	Polyethylene glycol E 400/ethanol (1:1)
GLP	Yes
Guideline	OECD guideline 412 (1981)
Deviation	The number of animals/test group is low; a minimum of 5/sex/dose are recommended in the current OECD guidance (2017). The number of concentrations is low; at least 3 test concentrations are recommended in the current OECD guidance (2017). Long post exposure observation period. The conditions given by the guideline were adapted to the special aim of examining the cataract inducing potential of the active ingredient administered via inhalation.
Impact of deviations	Minor – these deviations are minimal and are not considered to affect the validity of the study.
Acceptable	Acceptable with respect to examining the cataract inducing potential of the active ingredient
NOAEL	Cataract development in cats: 309 mg/m^3 air (the top concentration)
Effects at the LOAEL	N/A

Methods
Groups of 4 male and 4 female 'Forest of Dean' bred cats were whole-body exposed to mean analytical concentrations of 61 and 309 mg/m^3 air (maximum technically producible concentration) of tebuconazole aerosol. Vehicle control: polyethylene glycol E 400/ethanol 1:1, positive control: KNJ 0953 – 30 mg/m^3 air. Exposure time: four weeks (6 hours/day, 5 days/week), post-exposure observation period: 15 weeks.

During the weeks of exposure and the observation period body weights, clinical signs, mortality and ocular findings were recorded. At termination gross pathological and histopathological examinations were performed.

Results
Two low dose males died during the study (death not attributed to test substance). There were no findings at the clinical examinations. The body weight development was not affected in any concentration group.

Table 6.3-47. Incidences of cataracts

Sex	**Vehicle control**	**Positive control**	**Tebuconazole (mg/m^3 air)**	
			61	**309**
Male	1/4	4/4	1/2	0/4
Female	0/4	4/4	0/4	0/4

Table 6.3-48. Ocular findings at the end of the observation period (except for cataracts)

Findings	**Sex**	**Vehicle control**	**Positive control**	**Tebuconazole (mg/m^3 air)**	
				61	**309**
Yellow-tinged spots in lens fissure area	M F	1/4 0/4	0/4 0/4	0/2 0/4	0/4 3/4
Traced lens fissures	M F	0/4 1/4	0/4 0/4	0/2 0/4	0/4 1/4

Enhanced lens fissures	M F	1/4 0/4	0/4 1/4	0/2 0/4	1/4 2/4
White-tinged structures in vitreous humour	M F	1/4 0/4	0/4 0/4	0/2 0/4	0/4 0/4
Corneal opacity	M F	0/4 1/4	0/4 0/4	0/2 0/4	0/4 1/4
Dimples on cornea	M F	0/4 0/4	0/4 0/4	0/2 2/4	1/4 0/4
Blood vessel residues in anterior chamber	M F	0/4 0/4	0/4 0/4	0/2 0/4	1/4 0/4

M = Males
F = Females
n/n = number of animals with findings/number of surviving animals

At necropsy it was found that the two male animals from the low concentration group, which died intercurrently had thickening of the urinary bladder wall, mucous membrane inflammation, haemorrhage, and haemorrhagic urine in the bladder. At the histopathological examinations cataracts were found in all animals in the positive control group (Table 6.3-47). Only one male with cataracts was observed in treated animals (at 61 mg/m^3 air) and one male with cataracts was observed in the vehicle control group. Other ocular findings were not evident in the positive control group (Table 6.3-48). Whereas a range of other ocular findings were seen in animals in the top dose; while most findings were only seen in 1/8 animals yellow-tinged spots in lens fissure area was seen in 3/4 females in the high dose.

Conclusion
Under the conditions of this inhalation 4-week (6 hours/day, 5 days/week) study in cats tebuconazole shows no cataract inducing potential at concentrations up to 309 mg/m^3 air. The NOAEL for cataract development in cats is 309 mg/m^3 air. This is the same NOAEL value agreed during the first review of tebuconazole.

B.6.3.4.4. *Sub-acute inhalation study in dogs – study to investigate cataracts*

Previous evaluation	In Tebuconazole DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.3.4.4/01
Study title	HWG 1608 - (c.n. tebuconazole, proposed) - Subacute inhalation toxicity to dogs - study for cataracts
Test substance	(HWG 1608) Tebuconazole
Purity (%) **Batch no.**	97.1 816996033
Test animals	Female beagle dogs of the strain Bor:Beag
Groups	4/dose. Control group consisted of 4 untreated female dogs
Dose	163 and 914 mg/m^3 air (maximum technically producible concentration), 4 hours/day, 5 days/week for 6 weeks
Route	Head-nose only exposure to respirable aerosol (MMAD 1.4 µm; 90% particles ≤ 3 µm)
Vehicle	Polyethylene glycol:ethanol 1:1
GLP	Yes
Guideline	The technical methodology of the study based on OECD guideline no. 412 (1981), and EC 84/449/EWG B.8
Deviation	The number of animals/test group is low and representing one sex only; a minimum of 5/sex/dose are recommended in the current OECD guidance (2017). The number of doses is low; at least 3 test concentrations are recommended in the current OECD guidance (2017). Long post-exposure observation period. The conditions given by the guideline were adapted to the special aim of examining the cataract inducing potential of the active ingredient

	administered via inhalation.
Impact of deviations	Minor – these deviations are minimal and are not considered to affect the validity of the study.
Acceptable	Acceptable with respect to examining the cataract inducing potential of the active ingredient
NOAEL	163 mg/m^3 (approx. 8 mg/kg bw/day)
Effects at the LOAEL	Based on clinical signs in the high dose group (914 mg/m^3; approx. 44 mg/kg bw/day) - salivation, cough noises; transient loss of appetite.

Methods
Groups of 4 female beagle dogs were head-nose only exposed to target concentrations of 150 and 800 mg/m^3 air (analytical concentration 163 and 914 mg/m^3 air) of tebuconazole aerosol. No vehicle control was included. 4 untreated female dogs served as control group. Exposure time: 6 weeks (4 hours/day, 5 days/week), post-exposure observation period: 8 weeks.

During the weeks of exposure and observation period body weights, clinical signs, mortality and ocular findings, reflexes, food intake, lung function tests, blood examinations were performed. Measurements of the blood gases and the acid-base status were performed once near the end of exposure. At termination organ weights, gross pathological and histopathological examinations were performed.

Results
Treatment was tolerated without mortality. Reported clinical signs were salivation, cough noises (reversible within 2 hours after exposure); transient loss of appetite within the first two weeks of treatment in the dogs exposed to 914 mg/m^3 (the top concentration). In the reflex test there were no treatment-related effects. A slight (statistically significant) drop in body temperature was recorded in both groups of exposed animals and was explained by the laboratory as due to ethanol (vehicle used)-inhalation (central nervous system depression). There was statistically significantly decreased body weight gain in both treated groups during the last 3 weeks of exposure, which was not related to treatment. This was discussed by the performing laboratory to be due to administration of high concentrations of vehicle. This would need verification by inclusion of a vehicle control group rather than a control group of untreated animals. In the lung function test, the mean minute volume was marginally decreased after exposure in both treatment groups.

The analytical determination of tebuconazole in the blood showed that the mean serum level rose with the aerosol concentration indicating that the exposure conditions were adequate. No evidence was found for substance accumulation in the serum of dogs. The mean ethanol blood levels increased during the exposure.

The ophthalmic examinations showed no effects, which could be related to treatment.

There were no deviations in organ weights or other gross pathological changes at necropsy. No histopathological changes were recorded, which could be related to inhalational treatment with tebuconazole.

Conclusion
No cataract inducing potential of inhalational administration of tebuconazole is found in female beagle dogs at aerosol analytical concentrations of 163 and 914 mg/m^3 air (approx. 44 mg/kg bw/day assuming a breathing rate of 2 L/min and a body weight of 10 kg) for 4 hours/day, 5 days/week during 6 weeks. It is noted that although eye alterations were seen in dogs by the oral route (B.6.3.2.2/01) these occurred only at the high dose of approx. 212 mg/kg bw/day. Therefore, the absence of cataracts in this study conducted up to 44 mg/kg bw/day is not inconsistent with the oral study. NOAEL is found to be 163 mg/m^3 (approx. 8 mg/kg bw/day) based on clinical signs of toxicity in the high concentration group (914 mg/m^3). This is the same NOAEL value agreed during the first review of tebuconazole.

B.6.3.5. Summary of short-term toxicity

The oral short-term toxicity of tebuconazole has been investigated in a range of guideline compliant studies conducted in the rat, mouse and dog, with durations from 28-days to 12-months. There is also a dermal 3-week study in rabbits and inhalation studies in rats, cats and dogs. No publications of relevance to short-term toxicity have been identified from the open literature.

The following key conclusions were obtained from the evaluation of the short-term toxicity information:

- In studies in rats, mice, dogs and cats up to 12 months' duration, the target organs were the adrenal cortex, liver, spleen and eyes
- Classification for repeated-dose toxicity is not required.
- The data requirements of Regulation 283/2013 have been met.

Study	Dose range tested (mg/kg bw/d)	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d)	Effects at the LOAEL	Study reference
Oral 28-day study					
In rats					
28-day study. Oral gavage. + 4-week recovery. OECD 407 (1995). Non-GLP. Tebuconazole Batch 16001/83 Purity 97.0 % Rat. Wistar. Bor:WISW. Male and female. 20/sex/group.	0, 30, 100 and 300	30	100	≥ 100 mg/kg bw/d: ↓ red blood cell parameters (M/F), ↑rel. liver weights (M/F) and microsomal enzyme activities (M), ↑ abs. & rel. spleen weight (F), moderate to severe sideropenia in the spleen (F)	B.6.3.1.1/01
In mice					
Range-finding study (4-week) Oral, dietary. Non-GLP. Supporting study only. Doses of 0, 20, 60 and 180 ppm were proposed for the chronic toxicity feeding study in mice. Tebuconazole Mixed batch with fl. No. 132 Purity 96.9 % Mouse. Bor: NMRI (SPF Han). Male and female. 5/sex/group.	0, 125, 500 and 2000 ppm. Equivalent to: M/F: 0/0, 12.6/14.1, 47.0/53.2, 181.7/ 236.0 mg/kg bw/d.	Not established.	125 ppm Equivalent to 13 and 14 for males and females respectively.	≥ 12.6/14.1 mg/kg bw/d: ↑ liver weight (F), increased lipid accumulation in hepatocytes (M/F).	B.6.3.1.2/01
Range-finding study (8-week) Oral, dietary. Non-GLP. Supporting study	0, 500 and 2000 ppm Equivalent to: M/F: 0/0, 82/114,	Not established.	500 ppm Equivalent to 82 and 114 for males and	≥ 82/114 mg/kg bw/d: ↑ liver weight (M/F); slight to moderate liver cell degeneration (M/F), ↑slight vacuole formation and	B.6.3.1.2/02

Study	Dose range tested (mg/kg bw/d)	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d)	Effects at the LOAEL	Study reference
only. Doses of 0, 20, 60 and 180 ppm were proposed for the chronic toxicity feeding study in mice. Tebuconazole Mixed batch with fl. No. 132 Purity 96.9 % Mouse. Bor: NMRI (SPF Han). Male and female. 5/sex/group.	329/454 mg/kg bw/d.		females respectively	moderately increased fat content in the liver cells (M/F), ↑slight to moderate lipid content in adrenal cortex cells (M), ↑ serum iron levels.	
Oral 90-day study					
In rats					
Sub-chronic (13-week). Oral, dietary. OECD 408. GLP. Tebuconazole Batch 16007/83 Purity 98.2 % Rat Wistar (BOR:WISW) Male and female. 10/sex/group.	0, 100, 400 and 1600 ppm Equivalent to: M/F: 0/0, 8.6/10.8, 34.8/46.5, 171.7/235.2 mg/kg bw/d	100 ppm Equivalent to 9 and 11 for males and females respectively	400 ppm Equivalent to 35 and 47 for males and females respectively	≥ 34.8/46.5 mg/kg bw/d: ↓ body weight (M), very slight histopathological changes in adrenals (M/F).	B.6.3.2.1/01
In Dog					
90-day study Oral, dietary. OECD 409 (1981). GLP. Tebuconazole Batch 16007/83 Purity 98.2 % Dog Beagle (bor:beag) Male and female. 4/sex/ group.	0, 200, 1000 and 5000 ppm. Equivalent to: 8.5, 41 and 212 mg/kg bw/d.	1000 ppm Equivalent to 41 mg/kg bw/d.	5000 ppm Equivalent to 212 mg/kg bw/d.	212 mg/kg bw/d: lens degeneration (opacity) (M/F), ↓ food consumption & bodyweight (M/F), ↑ thrombocyte counts (M/F), anisocytosis (M/F), ↑ spleen weights (M/F), ↑ hemosiderosis in spleen and liver (M/F), ↑ adrenal vacuolation (F).	B.6.3.2.2/01
Oral 12-month study					
12-month Oral, dietary.	0, 40, 200 and 1000/2000 ppm	40 ppm	200 ppm	≥ 8 mg/kg bw/d: lens alterations (F), gross	B.6.3.3.1/01

Study	Dose range tested (mg/kg bw/d)	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d)	Effects at the LOAEL	Study reference
OECD 452. GLP. Tebuconazole Mixed batch Fl. 132 Purity 96.9 % Dog Beagle (bor:beag) Male and female. 4/sex/ group.	Equivalent to: 1.6, 8 and 47.3 mg/kg bw/d.	Equivalent to 1.6 mg/kg bw/d.	Equivalent to 8 mg/kg bw/d.	pathological changes in liver (F), histopathological changes in adrenals (F).	
12-month Oral, dietary. OECD 452. GLP. Tebuconazole Batch 16013/86 Purity 96 % Dog Beagle (bor:beag) Male and female. 4/sex/ group.	0, 100, 150 ppm Equivalent to: 3 and 4.4 mg/kg bw/d	100 ppm Equivalent to 3 mg/kg bw/d.	150 ppm (top dose) Equivalent to 4.4 mg/kg bw/d.	Hypertrophy of the adrenal zona fasciculata (M/F)	B.6.3.3.1/02
Dermal study					
Rabbit					
Subacute (3-week) dermal study of toxicity to rabbits, limit test. OECD 410. GLP. Tebuconazole Batch 16012/86 Purity 97.4 % Rabbit New Zealand White rabbits, strain HC:NZW Male and female. 5/sex/group.	0, 1000	1000	-	There were no systemic effects at 1000 mg/kg bw, but local effects possibly due to mechanical irritation	B.6.3.4/01
Inhalation study					
Rat					
3-week study (inhalation 6 hours/day, 5 days/week).	0, 1.2, 10.6, 155.8 mg/m^3 air (analytical concentrations)	10.6 mg/m^3 air	155.8 mg/m^3 air	Slight clinical symptoms (M/F) and liver enzyme induction (increased N-demethylase activity in the liver) (M/F).	B.6.3.4.2/01

Study	Dose range tested (mg/kg bw/d)	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d)	Effects at the LOAEL	Study reference
OECD 412 (1981). GLP. Tebuconazole Batch 16001/83 Purity 96.2 % Rat Wistar rats, strain Bor:WISW III. 5/sex/group.					
Cat					
28-day study (inhalation 6 hours/day, 5 days/week). Similar to OECD 412 (focus on cataract development), GLP. Tebuconazole Batch 16013/86 Purity 95.8 % Cat "Forest of Dean" breed. 4/sex/group.	0, 61, 309 mg/m^3 air (analytical concentrations)	With respect to cataract development: 309 mg/m^3 air	-	No cataract inducing potential was identified.	B.6.3.4.3/01
Dog					
6-week study (inhalation 4 hours/day, 5 days/week). Similar to OECD 412 (focus on cataract development). GLP Tebuconazole Batch 816996033 Purity 97.1 % Dog Beagle (bor:beag) Female 4/sex/group	163, 914 mg/m^3 air (high concentration equivalent to about 100 mg/kg bw/d). Cataract development 914 mg/m^3 air	163 mg/m^3 air	914 mg/m^3 air -	Clinical signs -salivation, tussive noises; transient loss of appetite. No cataract development	B.6.3.4.4/01

Oral
Rat

The short-term toxicity of tebuconazole via the oral route was investigated in the rat in a standard 28-day oral gavage study and 90-day feeding study covering a dose range of 8.6 – 235 mg/kg bw/d. Increased mortality was seen at the highest doses (171 and 235 mg/kg bw/d in males and females respectively) in the 90-day study. Predominant findings in the rat in both studies were effects on the liver and spleen. In the 28-day study decreased red blood cells were also seen. After 90 days effects on the adrenal cortex were identified.

Increases in absolute and relative liver weights were seen from 100 mg/kg bw/d in the 28-day study but no increase was observed in the 90-day study. Furthermore, effects on some clinical-chemistry parameters, which were mainly indicative of liver damage, as well as histopathological effects in the liver, were seen at 300 mg/kg bw/d in the 28-day study. In both studies, liver enzyme induction was observed, from 100 mg/kg bw/d in the 28-day study and from 35 mg/kg bw/d in males in the 90-day study.

In the spleen, increases in absolute and relative weights were seen from 100 mg/kg bw/d in the 28-day study. In addition, histopathological effects in the spleen were seen at 300 mg/kg bw/d and sideropenia was seen in females at 100 mg/kg bw/d in the 28-day study. In the 90-day study, hemosiderin accumulation in the spleen was seen at 235 mg/kg bw/d in females, the highest dose tested.

Effects on some haematological parameters (e.g. haematocrit values, haemoglobin content and MCV were decreased) were seen only in the 28-day study from 100 mg/kg bw/d.

In the adrenal cortex, increased vacuoles in the zona fasciculata was seen from 47 mg/kg bw/d in females and 172 mg/kg bw/d in males in the 90-day study.

It is noted that there were some inconsistencies in the observations made between these studies which may be reflective of the dosing regimen (gavage versus dietary) and duration of exposure.

Overall, taking into account the full range of observations, **the lowest relevant subchronic NOAEL in the rat was 9 mg/kg bw/d**. The LOAEL was 47 mg/kg bw/d based on slight histopathological changes in the adrenal cortex in females in the 90-day dietary study.

Mouse
The short-term toxicity of tebuconazole via the oral route was investigated in the mouse in two limited 4- and 8-week range-finding feeding studies.

The main target tissue was the liver. Absolute and relative liver weights were increased in females at 14 mg/kg bw/d and above after 28 days and 82 mg/kg/day (the lowest dose tested) after 56 days. Liver weight increases in males were seen at 181 mg/kg/day (highest dose) but not 47 mg/kg/day after 28 days and 114 mg/kg/day (lowest dose) after 56 days, possibly indicting an increasing severity of response with duration of treatment.

In addition, histopathological findings (such as pale, patchy, lobulated livers and liver degeneration) were seen at 53 mg/kg bw/d in females and at 182 mg/kg bw/d in males in the first study; and at 82 and 114 mg/kg bw/d in males and females respectively in the second study. An increased lipid accumulation in the hepatocytes from 13 and 14 mg/kg bw/d (the lowest dose tested) in males and females respectively and above was also seen in the first study. Induction of microsomal enzyme systems was seen in the liver from 31 mg/kg bw/d in males and females in the second satellite study.

Effects on the spleen (pigment deposition) were seen at the top dose of 329 and 454 mg/kg bw/d in males and females respectively in the 8-week study.

Adrenal cortex cells showed an increased lipid content from 82 mg/kg bw/d in males following 8-weeks of dosing.

A subchronic NOAEL in the mouse could not be established based on these limited range-finding studies as effects were seen at the lowest tested doses; however, these studies are useful as supporting information on potential target tissues.

Dog
The repeated dose oral toxicity of tebuconazole was investigated in the dog in 90-day and two 1-yr feeding studies covering a dose range of 1.6 – 212 mg/kg bw/d. The main target organs were the liver, spleen, eyes and adrenals.

Liver histopathology (hemosiderosis and/or increased lobulation) was seen at 212 mg/kg bw/d in the 90-day study and in the first 1-yr study at 8 mg/kg bw/d.

Spleen weights and histopathological changes (hemosiderosis) were seen at 212 mg/kg bw/d, the highest dose tested, in the 90-day study. Increased spleen siderin content was seen at 47.3 mg/kg bw/d, the highest dose tested, in one of the 1-yr studies.

In the eye, ophthalmoscopic alterations (lens opacity) were observed at 212 mg/kg bw/d in the 90-day study; this finding was confirmed in the first 1-yr study, where eye lesions were seen at 8 mg/kg bw/d and above.

In the adrenal cortex, increase in vacuole formation was seen at 212 mg/kg bw/d in the 90-day study; this was also seen in the second 1-yr study, at 4.4 mg/kg bw/d, the highest dose tested.

Haematological and clinical-chemistry parameters were observed in all studies in dogs.

The lowest sub-chronic NOAEL, from the first 1-yr study in the dog, was 1.6 mg/kg bw/d; the LOAEL in this study was 8 mg/kg bw/d, based on effects on the eye (lens), liver and vacuoles in the adrenal cortex. However, in the second 1-yr study in the dog, the highest NOAEL was 3 mg/kg bw/d, with a LOAEL of 4.4 mg/kg bw/d, based on hypertrophy of the adrenal zona fasciculate. Since the second study provides the highest NOAEL which lies below the lowest LOAEL in this relevant species and study type, **the most reliable sub-chronic NOAEL in the dog was 3 mg/kg bw/d.**

Dermal

In a 3-week dermal study in rabbits no systemic effects were recorded at the limit dose of 1000 mg/kg bw/d (the only dose tested). Minimal local irritation was considered due to mechanical irritation. **The NOAEL is 1000 mg/kg bw/d, the highest and only dose level in the study**.

Inhalation

In rats treated nose-only by inhalation to a respirable an aerosol of tebuconazole at concentrations of 1.2 – 155.8 mg/m^3 for three weeks, increased N-demethylase activity in the liver was the only effect observed. This is considered as an adaptive response of no toxicological significance. The RMS therefore concludes that there was **no systemic toxicity at the highest concentration tested of 155.8 mg/m^3 air**.

Cats (whole body) and dogs (head and nose only) were treated by inhalation to respirable aerosols of tebuconazole at concentrations of 61 – 914 mg/m^3 for four (cats) or six (dogs) weeks. This was to examine the cataract-inducing potential seen in dietary studies in dogs via the inhalation route of exposure. Cataract formation was not increased but the treatment caused body weight depression in the treated dogs at 914 mg/m^3 in dogs. **Administration of ≤309 mg/m^3 to cats for 4 weeks did not lead to cataract formation or any other indications of toxicity. The overall no observable effect concentration for the dogs was 163 mg/m^3 air.**

Conclusion

The oral short-term toxicity of tebuconazole has been investigated in a range of studies conducted in the rat, mouse and dog. There is also a dermal 3-week study in rabbits and inhalation studies in rats, cats and dogs. No publications of relevance to short-term toxicity have been identified from the open literature.

Several effects were observed consistently between species; the main target organ of toxicity was the liver, with increases in liver weights, effects on some clinical-chemistry parameters (indicative of liver damage) and changes to liver histopathology consistently seen in all three species. Adverse effects on the spleen, including changes to its histopathology and adverse effects on the adrenals including changes to its histopathology (e.g. increase in lipid content and vacuolation) were also seen in all three species. Adverse effects in the eye, such as lens degeneration and eye lesions were consistently seen in dog studies, but not in rats and mice. There is no evidence that eye effects are of no relevance to humans. Adverse effects on body weight development and on some haematological parameters were observed in the rat and dog but not in mice.

The dog was the most sensitive species, with adverse effects being observed in more tissues (i.e. additionally in

the eye) and at lower dose levels than in other species (i.e. observed LOAELs of 4.4 mg/kg bw/d in the dog compared to 13 mg/kg bw/d in mice and 47 mg/kg bw/d in the rat).

The lowest relevant NOAEL from all the available short-term toxicity studies therefore was 1.6 mg/kg bw/d from the 12-month study in the dog. The LOAEL in this study was 8 mg/kg bw/d based on lens alterations, increased incidence of livers with lobulation and vacuoles in cells of zona fasciculata of the adrenals (in females).

B.6.4. GENOTOXICITY

The genotoxic potential of tebuconazole has been investigated in a series of *in vitro* and *in vivo* studies.

Bayer task force (BTF) provided a package of genotoxicity studies including nine *in vitro* and three *in vivo* studies. These studies were evaluated in the original DAR (2006), peer reviewed at EU level (EFSA Conclusion, 2008) and considered to be acceptable. They are re-summarised and re-evaluated below. In addition three new *in vitro* studies (bacterial reverse mutation assay, mammalian cell gene mutation, *in vitro* micronucleus test) and one *in vivo* (micronucleus test) have been provided for the purpose of renewal and have not previously been considered.

EU Tebuconazole Task Force (OTF) have provided a package of three *in vitro* bacterial reverse mutation assays and one *in vivo* study (micronucleus test). These studies were provided for the purpose of renewal and have not previously been considered.

According to Regulation (EU) 283/2013, photo-mutagenicity testing is not required for substances with a UV/VIS molar extinction/absorption coefficient less than 1000 L x mol^{-1} x cm^{-1}. There is no relevant absorption in the range 290 - 700 nm and the ultraviolet/visible molar extinction/absorption coefficient of the active substance is less than 10 L x mol^{-1} x cm^{-1} (see chemistry evaluation section B.2.4). Photo-mutagenicity testing is therefore not required for tebuconazole. The RMS also notes that there is currently no OECD test guideline available for photo-mutagenicity testing and Regulation (EU) 283/2013 does not provide any guidance on suitable methods.

B.6.4.1. *In vitro* studies

A number of *in vitro* genotoxicity studies were evaluated in the original DAR (2006), peer reviewed at EU level (EFSA Conclusion, 2008) and considered to be acceptable. These are re-summarised and re-evaluated below. In addition, new *in vitro* micronucleus study, reverse mutation assays, and a mammalian cell gene mutation study have been submitted for the purpose of renewal and have not previously been considered. An *in vitro* photogenotoxicity study is not required.

B.6.4.1.1. *Pol Test on Escherichia coli*

One non-standard Pol test in *Escherichia coli* is available. This was considered in the original DAR (2006).

Previous evaluation:	In Tebuconazole DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.4.1.1/01
Study title	HWG 1608 - Pol Test on *E. coli* to evaluate for harmful effects on DNA
Test substance	(HWG 1608) Tebuconazole
Purity (%) **Batch no.**	97.1 16001/83
Test system	*Escherichia coli* strains: (K12)p 3478 (repair deficient) and W 3110 (repair capable)
Groups	5 test concentrations plus appropriate positive and negative controls.
Concentration	0, 625, 1250, 2500, 5000, and 10000 µg/plate. Positive control: Methyl methane sulphonate 10 µL/plate. Negative control: Chloramphenicol 30 µg/plate
Vehicle	DMSO
GLP	No
Guideline	n/a
Deviation	n/a

Acceptable	Acceptable – Supplementary, as a non-standard test
Result	Non-mutagenic in the Pol test at concentrations up to and including 10000 µg/plate, with or without metabolic activation,

Methods
Tebuconazole was tested in the pol test on *E. coli* (described by Rosenkranz and Leifer, 1980) at concentrations up to 10000 µg/plate dissolved in DMSO. Two *E. coli* strains, one repair deficient and one repair capable, were used. Methyl methane sulphonate (MMS) served as positive control and Chloramphenicol as negative control. Four plates were used per substance, concentration and strain, both with and without S-9 mix. The plates were incubated for 24 hours at 37ºC.

Results
No biologically relevant increase in difference in inhibition zone diameters (> 2 mm) was noted at any of the five concentrations used. This applied both to the tests with and without S-9 mix.

The positive control tests demonstrated the sensitivity of the system.

Conclusion
Tebuconazole was considered to be non-mutagenic in the Pol test at concentrations up to and including 10000 µg/plate, with or without metabolic activation, whether tested in a repair deficient or a repair capable strain of *Escherichia coli*. As this is a non-GLP, non-standard test, it is only considered supplementary information.

B.6.4.1.2. ***Bacterial reverse mutation test***

Seven Ames tests are available. Three, owned by Bayer, were considered in the original DAR (2006) and four (one by BTF and three by OTF) have been submitted for the purposes of renewal.

1)

Previous evaluation:	In Tebuconazole DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.4.1.2/01
Study title	*Salmonella*/microsome test to evaluate for point mutagenic effects *Salmonella*/microsome test using TA1538 to evaluate for point mutagenic effects
Test substance	(HWG 1608) Tebuconazole
Purity (%) **Batch no.**	96.6 1616001/86
Test system	*Salmonella typhimurium*: strains TA1535, TA1537, TA98, TA100, TA1538
Groups	Four plates per concentration and strain
Concentration	for TA1535, TA1537, TA98 and TA100: first test: 0, 37.5, 75, 150, 300, 600, 1200 and 2400 µg/plate repeat test: 0, 39.5, 59.3, 88.9, 133.3, 200, 300 and 450 µg/plate (due to the substance's toxicity lower doses were chosen for the repeat test) for TA1538: first and repeat test: 0, 39.5, 59.3, 88.9, 133.3, 200, 300 and 450 µg/plate
Vehicle	DMSO
GLP	Yes
Guideline	OECD 471
Deviation	The following deviations from the OECD-Guideline 471 (2016) occurred: - No tests were performed in *E. coli* or alternatively in S. typhimurium strain TA102 (to detect oxidising mutagens or cross linking agents)
Acceptable	Acceptable in a WoE approach. The limitations of this study are compensated by the availability of more modern Ames studies.
Result	Not mutagenic up to and including 450 µg/plate with or without metabolic activation in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 or TA1538

Methods
The mutagenicity of the test substance was evaluated with the *Salmonella*/microsome test, also termed the Ames Test. Four plates per dose and strain were used for mutants' counts, both with and without S-9 mix. Concentrations were tested up to bacteriostatic effects and deemed adequate.
Positive controls:
2-Aminoanthracene (3 µg/plate for all strains)
Sodium azide (10 µg/plate for TA1535)
Nitrofurantoin (0.2 µg/plate for TA100)
4-nitro-1,2-phenylene diamine (10 µg/plate for TA1537; 0.5 µg/plate for TA98 and TA1538)

Results
Concentrations up to and including 39.5 µg/plate did not cause any bacteriotoxic effects in all strains. At higher doses strong strain specific bacteriotoxic effects were observed, so this range could only be used to a limited extent up to 600 µg/plate for evaluation purposes.

Evidence of mutagenic activity for tebuconazole was not found. Neither a concentration-related doubling nor a biologically relevant increase of mutant count, in comparison with the negative controls, were observed. The positive controls had a marked mutagenic effect, as was seen by a biologically relevant increase of mutagenic colonies compared with the negative controls.

Conclusion
Overall, tebuconazole was not mutagenic in a *Salmonella typhimurium* reverse mutation assay (either the plate incorporation test and the pre-incubation experiment) up to cytotoxic concentrations (up to and including 450 µg/plate) in the presence and absence of metabolic activation.

2)

Previous evaluation:	None – submitted for the purpose of renewal (study owned by EU Tebuconazole Task Force)

Study ID	B.6.4.1.2/02
Study title	Reverse mutation assay using bacteria (*Salmonella typhimurium* and *Escherichia coli*) with Tebuconazole TGAI
Test substance	Tebuconazole TGAI
Purity (%) **Batch no.**	98.8 TBZ1003060
Test system	*Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537; *E. coli* WP2 uvrA
Groups	Triplicate for all treatments
Concentration	Experiment I: 3.16, 10.0, 31.6, 100, 316, 1000, 2500 µg/plate; in addition, only for TA 100 (with metabolic activation) and *E.coli* WP2 uvrA (with and without metabolic activation): 5000 µg/plate Experiment II: 1.00, 3.16, 10.0, 31.6, 100, 316, 1000 µg/plate (TA 98, TA 100, TA 1535, TA 1537); 31.6, 100. 316, 1000, 2500, 5000 µg/plate (*E.coli* WP2 uvrA)
Vehicle	DMSO
GLP	Yes
Guideline	OECD Guideline 471
Deviation	The following deviations from the OECD-Guideline 471 (2016) occurred: - None
Acceptable	Acceptable
Result	Tebuconazole was not mutagenic up to cytotoxic/limit concentrations in the presence and absence of metabolic activation.

Methods
Salmonella typhimurium strains TA1535, TA100, TA1537, TA98, and *Escherichia coli* WP2 uvrA were exposed to tebuconazole dissolved in dimethylsulfoxide (DMSO) in the presence and absence of metabolic activation (rat liver-derived S9 mix). The study design included the testing of appropriate positive, negative and solvent controls. In the plate incorporation assay the test substance was tested at concentrations ranging from 3.16 µg/plate up to the test limit concentration of 5000 µg/plate. The assay was repeated as a pre-incubation assay with concentrations

ranging from 1 µg/plate up to the test limit concentration of 5000 µg/plate. There were triplicate plates for all test substance, solvent (DMSO) control and positive control treatments

Positive controls
Without metabolic activation:
Sodium azide (TA 100, TA 1535)
4-nitro-o-phenylene-diamine (TA 98, TA 1537)
Methylmethanesulfonate (*E.coli* WP2 uvrA)

With metabolic activation:
2-aminoanthracene (TA 98, TA 100, TA 1535, TA 1537, *E.coli* WP2 uvrA)

HCD controls available but not clear exactly which mutagens were used for positive control

Results
In the plate incorporation assay tebuconazole produced bacteriotoxic effects in all strains used at concentrations of 316 µg per plate and higher (with and without metabolic activation) depending on particular test strain. Substance precipitation was observed at 5000 µg per plate only. There were no biologically relevant, concentration-related or reproducible mutagenic effects compared to solvent controls with and without S9 in any strain.

In the *Salmonella*/microsome test, using preincubation for 60 minutes at 37 °C, tebuconazole produced strain-specific bacteriotoxic effects from the concentration of 100 µg per plate and higher (with and without metabolic activation). Substance precipitation was observed at 5000 µg per plate. In agreement with the plate incorporation assay, there were no biologically relevant, concentration-related or reproducible mutagenic effects compared to solvent controls with and without S9 in any strain.

The positive controls induced the appropriate response, increasing the mutant counts compared to solvent controls, thus demonstrating the sensitivity of the test system and the activity of the S9 mix.

Conclusion
Overall, tebuconazole was not mutagenic in a guideline, modern *Salmonella typhimurium* reverse mutation assay (either the plate incorporation test and the pre-incubation experiment) up to cytotoxic/limit concentrations in the presence and absence of metabolic activation.

3)

Previous evaluation:	In Tebuconazole DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.4.1.2/03
Study title	HWG 1608 - *Salmonella*/microsome test to evaluate for point-mutagenic effect
Test substance	(HWG 1608) Tebuconazole
Purity (%) **Batch no.**	97.0 16001/83
Test system	Histidine-dependent *Salmonella typhimurium* strains TA1535, TA100, TA1537, and TA98
Groups	Four plates were used per substance (active ingredient and positive control), dose and strain both with and without S-9 mix.
Concentration	0, 20, 75, 100, 150, 300, 500, 600, 1200, 2500, and 12500 µg/plate.
Vehicle	DMSO
GLP	Yes, but no inspections were performed by the Quality Assurance Unit during the study period
Guideline	OECD Guideline 471
Deviation	The following deviations from the OECD-Guideline 471 (2016) occurred: - None
Acceptable	Acceptable in a WoE approach with more modern Ames studies.
Result	Non-mutagenic in the *Salmonella*/microsome assay, with and without metabolic activation when tested at concentrations from 20 to 12500 µg/plate

Method
Tebuconazole was tested in the *Salmonella*/microsome assay at concentrations up to 12500 µg/plate dissolved in DMSO with and without S-9-mix. The *Salmonella* strains were the histidine-requiring LT2 mutants TA1535, TA1537, TA100 and TA98. Cyclophosphamide (Endoxan) (145 (TA 1535) or 290 (TA 100) µg/plate), 2-aminoanthracene (3 (TA 1537 and TA 98) µg/plate) and trypaflavin (50 µg/plate) were used as positive controls. Four plates were used per substance (active ingredient and positive control), dose and strain, both with and without S-9 mix. The plates were incubated for 48 hours at 37ºC.
Results
Tebuconazole concentrations up to and including 150 µg/plate did not exert any toxic effects to the bacteria. At higher concentrations, the test compound had a strain related bacteriotoxicity both with and without S-9 mix so that this concentration-range was only of limited use for assessment up to 2500 µg/plate. The concentration level of 12500 µg/plate resulted in precipitation of the substance.

There were no indications of any mutagenic effect of tebuconazole. There was neither a concentration-related doubling nor a biologically relevant increase in the mutant counts in relation to the solvent controls.

The positive controls demonstrated the sensitivity of the test system and the activity of the S-9 mix.

Conclusion
Tebuconazole was found to be non-mutagenic in the *Salmonella*/microsome assay, with and without metabolic activation when tested at concentrations from 20 to 12500 µg/plate. Concentrations up to 2500 µg/plate were (partly) evaluable, due to cytotoxicity.

4)

Previous evaluation:	In Tebuconazole DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.4.1.2/04
Study title	HWG 1608 - Reverse mutation assay (*Salmonella typhimurium* and *Escherichia coli*)
Test substance	(HWG 1608) Tebuconazole
Purity (%) **Batch no.**	98.0 816096181
Test system	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and *Escherichia coli* strain WP2 uvrA
Groups	Three plates were evaluated for each test solution and control.
Concentration	In *S. typhimurium*: 15.625, 31.25, 62.5, 125, 250, and 500 µg/plate both +/- S9 mix In *E. coli*: 31.25, 62.5, 125, 250, 500, and 1000 µg/plate without S-9 mix 156.25, 312.5, 625, 1250, 2500, and 5000 µg/plate with S-9 mix
Vehicle	DMSO
GLP	Yes
Guideline	OECD Guideline 471
Deviation	The following deviations from the OECD-Guideline 471 (2016) occurred: - None
Acceptable	Acceptable
Result	Not found to induce base-pair or frameshift mutations in bacteria in the reverse mutation assay with or without metabolic activation.

Methods
Salmonella typhimurium strains TA1535, TA100, TA1537, TA98, and *Escherichia coli* WP2 uvrA were exposed to tebuconazole dissolved in dimethylsulfoxide (DMSO) in the presence and absence of metabolic activation (rat liver-derived S9 mix). The study design included the testing of appropriate positive, negative and solvent controls. The assay was performed as a pre-incubation assay with concentrations ranging from 15.625 µg/plate up to the test limit concentration of 5000 µg/plate. There were triplicate plates for all test substance, solvent (DMSO) control and positive control treatments and the pre incubation assay was performed twice.

Positive controls:

2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide:	0.1 µg/plate	(TA98, – S-9 mix)
	0.01 µg/plate	(TA100, – S-9 mix)
	0.04 µg/plate	(WP2 uvrA; – S-9 mix)
2-aminoanthracene:	0.5 µg/plate	(TA 98; + S-9 mix)
	1 µg/plate	(TA100; + S-9 mix)
	2 µg/plate	(TA1535, TA 1537; + S-9 mix)
	20 µg/plate	(WP2 uvrA; + S-9 mix)
sodium azide:	0.5 µg/plate	(TA1535; – S-9 mix)
9-aminoacridine:	80 µg/plate	(TA1537; – S-9 mix)

Results
The top concentrations demonstrated growth inhibition. No marked increase in the number of revertant colonies was observed at any test concentration. A clear increase in the number of revertant colonies was observed in the positive control tests, demonstrating that the study had been performed under appropriate conditions.

Conclusion
Tebuconazole was not found to induce base-pair or frameshift mutations in bacteria in the reverse mutation assay with or without metabolic activation up to the limit concentration.

5)

Previous evaluation:	None – submitted for the purpose of renewal (study owned by Bayer task force)

Study ID	B.6.4.1.2/05
Study title	Tebuconazole: *Salmonella typhimurium* reverse mutation assay
Test substance	Tebuconazole
Purity (%) **Batch no.**	95.7 % (w/w) Specification No: 102000006666 Batch No. 2015-005886
Test system	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA102
Groups	Three plates were evaluated for each test solution and control.
Concentration	Plate incorporation assay: All strains: 3, 10, 33, 100, 333, 1000, 5000 µg/plate Pre-incubation assay: TA 102: 10, 33, 100, 333, 1000, 2500, 5000 µg/plate Other strains: 3, 10, 33, 100, 333, 1000, 2500, 5000 µg/plate
Vehicle	DMSO
GLP	Yes
Guideline	OECD Guideline 471
Deviation	The following deviations from the OECD-Guideline 471 (2016) occurred: - None
Acceptable	Acceptable
Result	Not mutagenic in a guideline, modern *Salmonella typhimurium* reverse mutation assay up to cytotoxic/limit concentrations in the presence and absence of metabolic activation.

Methods
Salmonella typhimurium strains TA1535, TA100, TA1537, TA98, and TA102 were exposed to tebuconazole dissolved in dimethylsulfoxide (DMSO) in the presence and absence of metabolic activation (rat liver-derived S9 mix). The study design included the testing of appropriate positive, negative and solvent controls. In the plate incorporation assay the test substance was tested at concentrations ranging from 3 µg/plate up to the test limit concentration of 5000 µg/plate. The assay was repeated as a pre-incubation assay with concentrations ranging from 3 µg/plate up to the test limit concentration of 5000 µg/plate. There were triplicate plates for all test substance, solvent (DMSO) control and positive control treatments. Historical control data is available

Positive controls
Without metabolic activation:

Strain	Mutagen	Solvent	Conc.

TA 1535	sodium azide, NaN_3	deionised water	10 µg/plate
TA 1537	4-nitro-o-phenylene-diamine, 4-NOPD	DMSO	50 µg/plate
TA 98	4-nitro-o-phenylene-diamine, 4-NOPD	DMSO	10 µg/plate
TA 100	sodium azide, NaN_3	deionised water	10 µg/plate
TA 102	methyl methane sulfonate, MMS	deionised water	2.0 µL/plate

With metabolic activation:

Strain	**Mutagen**	**Solvent**	**Conc.**
TA 1535	2-aminoanthracene, 2-AA	DMSO	2.5 µg/plate
TA 1537			
TA 98			
TA 100			
TA 102			10.0 µg/plate

Results
Precipitation of the test item in the overlay agar of the incubated agar plates was observed from 1000 to 5000 µg/plate without S9 mix and from 2500 to 5000 µg/plate with S9 mix in both experiments. The undissolved particles had no influence on the data recording, but colonies were partly counted manually to account for this precipitation.

The plates incubated with the test item showed normal background growth up to 5000 µg/plate with and without S9 mix in all strains used. Toxic effects, evident as a reduction in the number of revertants (below the induction factor of 0.5), were observed at concentrations from 333 µg/plate depending on the strain used (Table 6.4-1).

Table 6.4-1. Summary of cytotoxicity assay

Strain	**Experiment I**		**Experiment II**	
	without S9 mix	**with S9 mix**	**without S9 mix**	**with S9 mix**
TA 1535	1000 – 5000	/	5000	5000
TA 1537	2500 – 5000	2500 – 5000	1000 – 5000	2500 – 5000
TA 98	2500 – 5000	2500 – 5000	1000 – 5000	2500 – 5000
TA 100	333 – 5000	1000 – 5000	333 – 5000	333 – 5000
TA 102	/	/	5000	/

/ = No toxic effects (induction factor < 0.5)

In the plate incorporation assay tebuconazole produced bacteriotoxic effects in strains TA 1535, 1537, 98 and 100, at concentrations of 333 µg per plate and higher (without metabolic activation) and TA 1537, 98 and 100 at concentrations of 1000 µg per plate and higher (with metabolic activation). There were no biologically relevant, concentration-related or reproducible mutagenic effects compared to solvent controls with and without S9 in any strain.

In the *Salmonella*/microsome test, using preincubation for 60 minutes at 37 °C, tebuconazole produced strain-specific bacteriotoxic effects from the concentration of 333 µg per plate and higher (with and without metabolic activation). In agreement with the plate incorporation assay, there were no biologically relevant, concentration-related or reproducible mutagenic effects compared to solvent controls with and without S9 in any strain.

The appropriate positive mutagen controls induced the appropriate response, increasing the mutant revertant colony counts compared to solvent controls, thus demonstrating the sensitivity of the test system and the activity of the S9 mix.

Table 6.4-2. Summary of mean values (mutant counts) with/without S9 Mix (plate incorporation assay)

Metabolic Activation	Test Group	Dose Level (per plate)	Revertant Colony Counts (Mean ±SD) TA 1535	TA 1537	TA 98	TA 100	TA 102
Without Activation	DMSO		13 ± 1	11 ± 2	25 ± 3	173 ± 14	476 ± 23
	Untreated		13 ± 3	13 ± 2	40 ± 2	182 ± 20	527 ± 16
	Tebuconazole	3 µg	14 ± 3	10 ± 5	25 ± 4	192 ± 5	485 ± 5
		10 µg	11 ± 1	10 ± 6	32 ± 4	169 ± 23	509 ± 22
		33 µg	12 ± 4	12 ± 6	28 ± 3	171 ± 2	514 ± 30
		100 µg	8 ± 3	9 ± 2	34 ± 8	176 ± 17	518 ± 8
		333 µg	9 ± 3	10 ± 5	26 ± 9	43 ± 7	499 ± 20
		1000 µg	5 ± 1 P	8 ± 4 M P	26 ± 3 P	20 ± 1 P	453 ± 31 P
		2500 µg	1 ± 1 M P	2 ± 1 P M	5 ± 1 P	1 ± 0 P	353 ± 26 P
		5000 µg	0 ± 1 M P	0 ± 1 P M	0 ± 0 P	0 ± 0 P	349 ± 33 P
	NaN_3	10 µg	1198 ± 80			2209 ± 24	
	4-NOPD	10 µg			545 ± 66		
	4-NOPD	50 µg		73 ± 8			
	MMS	2.0 µL					5131 ± 400
With Activation	DMSO		12 ± 2	16 ± 5	40 ± 7	177 ± 22	635 ± 6
	Untreated		15 ± 1	11 ± 4	43 ± 5	175 ± 11	667 ± 7
	Tebuconazole	3 µg	10 ± 2	12 ± 4	48 ± 1	131 ± 15	722 ± 15
		10 µg	11 ± 1	18 ± 6	44 ± 7	125 ± 4	716 ± 16
		33 µg	7 ± 3	12 ± 6	36 ± 9	146 ± 25	718 ± 27
		100 µg	11 ± 5	9 ± 2	30 ± 9	129 ± 23	732 ± 25
		333 µg	10 ± 1	13 ± 1	34 ± 1	89 ± 27	705 ± 22
		1000 µg	14 ± 2	12 ± 2	25 ± 3	9 ± 1	750 ± 11
		2500 µg	8 ± 2 M P	6 ± 2 P M	5 ± 2 P M	4 ± 2 P	740 ± 22 P
		5000 µg	7 ± 2 M P	2 ± 1 P M	1 ± 1 P M	0 ± 1 P M	634 ± 34 P
	2-AA	2.5 µg	230 ± 81	323 ± 28	4165 ± 267	4685 ± 211	
		10.0 µg					1383 ± 119

NaN3 sodium azide P Precipitate
2-AA 2-aminoanthracene M Manual count
MMS methyl methane sulfonate
4-NOPD 4-nitro-o-phenylene-diamine

Table 6.4-3. Summary of mean values (mutant counts) with/without S9 Mix (pre-incubation assay)

Metabolic Activation	Test Group	Dose Level (per plate)	Revertant Colony Counts (Mean ±SD) TA 1535	TA 1537	TA 98	TA 100	TA 102
Without Activation	DMSO		10 ± 4	9 ± 3	27 ± 6	120 ± 16	461 ± 19
	Untreated		13 ± 3	15 ± 2	37 ± 5	191 ± 13	502 ± 34
	Tebuconazole	3 µg	8 ± 2	11 ± 5	33 ± 2	119 ± 3	
		10 µg	9 ± 2	9 ± 2	28 ± 7	127 ± 10	497 ± 27
		33 µg	8 ± 1	12 ± 3	31 ± 7	127 ± 8	478 ± 22
		100 µg	11 ± 2	9 ± 3	27 ± 9	79 ± 4	482 ± 46
		333 µg	9 ± 3	8 ± 4	15 ± 3	23 ± 6	446 ± 9
		1000 µg	9 ± 2 P	4 ± 2 P	3 ± 1 P M	3 ± 1 P	332 ± 33 P
		2500 µg	6 ± 2 P M	0 ± 1 P	1 ± 1 P M	2 ± 2 P	315 ± 13 P
		5000 µg	1 ± 1 P M	0 ± 0 P	0 ± 0 P M	0 ± 0 P	114 ± 15 P M
	NaN_3	10 µg	1113 ± 128			1955 ± 120	
	4-NOPD	10 µg			471 ± 22		
	4-NOPD	50 µg		71 ± 10			
	MMS	2.0 µL					3767 ± 417

Metabolic Activation	Test Group	Dose Level (per plate)	Revertant Colony Counts (Mean ±SD)				
			TA 1535	TA 1537	TA 98	TA 100	TA 102
With Activation	DMSO		18 ± 3	16 ± 5	38 ± 9	119 ± 12	636 ± 7
	Untreated		18 ± 4	17 ± 3	46 ± 2	175 ± 6	621 ± 19
	Tebuconazole	3 µg	15 ± 6	18 ± 3	46 ± 7	116 ± 9	
		10 µg	18 ± 2	19 ± 3	49 ± 6	100 ± 9	580 ± 52
		33 µg	16 ± 6	22 ± 5	36 ± 5	109 ± 17	536 ± 56
		100 µg	17 ± 8	22 ± 1	38 ± 4	108 ± 17	585 ± 20
		333 µg	14 ± 5	15 ± 2	44 ± 3	32 ± 4	625 ± 84
		1000 µg	9 ± 3	19 ± 2	48 ± 4	17 ± 6	514 ± 21
		2500 µg	11 ± 4 P	5 ± 2 P M	4 ± 2 P M	1 ± 1 P	478 ± 4 P
		5000 µg	5 ± 2 P M	3 ± 1 P M	1 ± 1 P M	0 ± 1 P M	380 ± 31 P
	2-AA	2.5 µg	301 ± 33	229 ± 35	3475 ± 650	3516 ± 159	
		10.0 µg					1335 ± 68

NaN3	sodium azide	P	Precipitate
2-AA	2-aminoanthracene	M	Manual count
MMS	methyl methane sulfonate		
4-NOPD	4-nitro-o-phenylene-diamine		

Conclusion
Overall, tebuconazole was not mutagenic in a guideline, modern *Salmonella typhimurium* reverse mutation assay (either the plate incorporation test or the pre-incubation experiment) up to cytotoxic/limit concentrations in the presence and absence of metabolic activation.

6)

Previous evaluation	None – submitted for the purpose of renewal (study owned by EU Tebuconazole Task Force)

Study ID	B.6.4.1.2/06
Study title	Tebuconazole technical batch no. 2013021806: Reverse Mutation Assay "Ames test" using *Salmonella typhimurium* and *Escherichia coli*
Test substance	Tebuconazole Technical
Purity (%) **Batch no.**	98.4 Batch No. 2013021806
Test system	*Salmonella typhimurium* strains TA 1535, TA 1537, TA 98 and TA 100 and *Escherichia coli* strain WP2 uvrA
Groups	Three plates were evaluated for each test solution and control.
Concentration	Experiment I: 1.5, 5, 15, 50, 150, 500, 1500, 5000 µg/plate Experiment II: 1.5, 5, 15, 50, 150, 500, 1500, 5000 µg/plate
Vehicle	DMSO
GLP	Yes
Guideline	OECD Guideline 471
Deviation	The following deviations from the OECD-Guideline 471 (2016) occurred: - none
Acceptable	Acceptable
Result	Tebuconazole technical was considered to be non-mutagenic under the conditions of the test.

Methods
Salmonella typhimurium strains TA1535, TA100, TA1537, TA98, and *Escherichia coli* WP2 uvrA were exposed to tebuconazole dissolved in dimethylsulfoxide (DMSO) in the presence and absence of metabolic activation (rat liver-derived S9 mix). The study design included the testing of appropriate positive, negative and solvent controls. In the plate incorporation assay the test substance was tested at concentrations ranging from 1.5 µg/plate up to the test limit concentration of 5000 µg/plate. The assay was repeated as a pre-incubation assay with concentrations ranging from 1.5 µg/plate up to the test limit concentration of 5000 µg/plate. There were triplicate plates for all

test substance, solvent (DMSO) control and positive control treatments. Historical control data is available.

Positive controls
Without metabolic activation:
N-ethyl-N'-nitro-N-nitrosoguanidine (TA 100, TA 1535, *E.coli* WP2 uvrA)
9-Aminoacridine (TA 1537)
4-Nitroquinoline-1-oxide (TA 98)

With metabolic activation:
2-aminoanthracene (TA 100, TA 1535, TA 1537, *E.coli* WP2 uvrA)
Benzo(a)pyrene (TA 98)

Results
The vehicle control plates gave counts of revertant colonies within the normal range. All of the positive control chemical used in the test induced marked increases in the frequency of revertant colonies, both with and without metabolic activation. Thus the sensitivity of the assay and the efficacy of the S9-mix were validated.

In the plate incorporation assay tebuconazole produced bacteriotoxic effects in all *Salmonella* strains used at concentrations of 1500 µg per plate and higher (with and without metabolic activation). No toxicity was noted to *E.coli* WP2 uvrA. No test item precipitate was observed on the plates at any concentrations tested in either the presence or absence of metabolic activation. There were no biologically relevant, concentration-related or reproducible mutagenic effects compared to solvent controls with and without S9 in any strain.

In the *Salmonella*/microsome test, using preincubation for 60 minutes at 37 °C, tebuconazole produced strain-specific bacteriotoxic effects at 500 µg/plate (TA 1535 and TA 1537), 1500 µg/plate (TA 100 and TA 98) and at 5000 µg/plate (WP2 uvrA). No test item precipitate was observed on the plates at any doses tested in either the presence or absence of metabolic activation. In agreement with the plate incorporation assay, there were no biologically relevant, concentration-related or reproducible mutagenic effects compared to solvent controls with and without S9 in any strain.

Conclusion
Overall, tebuconazole was not mutagenic in a guideline, modern *Salmonella typhimurium/Escherichia coli* reverse mutation assay (either the plate incorporation test or the pre-incubation experiment) up to cytotoxic/limit concentrations in the presence and absence of metabolic activation.

7)

Previous evaluation:	None – submitted for the purpose of renewal (study owned by EU Tebuconazole Task Force)

Study ID	B.6.4.1.2/07
Study title	Tebuconazole technical bacterial reverse mutation test
Test substance	Tebuconazole Technical
Purity (%) **Batch no.**	97.21 Batch No. 081001
Test system	*Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537; *E. coli* WP2 uvrA
Groups	Three plates were evaluated for each test solution and control.
Concentration	Experiment I: 5, 15, 50, 150, 500, 1500, 5000 µg/plate Experiment II: 50, 150, 500, 1500, 5000 µg/plate
Vehicle	DMSO
GLP	Yes
Guideline	OECD Guideline 471
Deviation	The following deviations from the OECD-Guideline 471 (2016) occurred: None
Acceptable	Acceptable
Result	Tebuconazole technical was considered to be non-mutagenic under the conditions of the test.

Methods
Salmonella typhimurium strains TA 1535, TA 100, TA 1537, TA 98, and *Escherichia coli* WP2 uvrA were exposed to tebuconazole dissolved in dimethylsulfoxide (DMSO) in the presence and absence of metabolic activation (rat liver-derived S9 mix). The study design included the testing of appropriate positive, negative and solvent controls. In the plate incorporation assay the test substance was tested at concentrations ranging from 5 µg/plate up to the test limit concentration of 5000 µg/plate. The assay was repeated as a pre-incubation assay with concentrations ranging from 50 µg/plate up to the test limit concentration of 5000 µg/plate. There were triplicate plates for all test substance, solvent (DMSO) control and positive control treatments. Historical control data is available.

Positive controls
Without metabolic activation:
Sodium azide (TA 100, TA 1535)
9-Aminoacridine (TA 1537)
2-Nitrofluorene (TA 98)
4-Nitroquinoline (*E. coli* WP2 uvrA)

With metabolic activation:
2-aminoanthracene (TA 100, TA 1535, *E.coli* WP2 uvrA)
Benzo(a)pyrene (TA 98, TA 1537)

Results
The vehicle control plates gave counts of revertant colonies within the normal range. All of the positive control chemicals used in the test induced marked increases in the frequency of revertant colonies, both with and without metabolic activation. Thus the sensitivity of the assay and the efficacy of the S9-mix were validated.
In the plate incorporation assay tebuconazole produced no bacteriotoxic effects in the tester strains used. No test item precipitate was reported on the plates at any doses tested in either the presence or absence of metabolic activation. There were no biologically relevant, concentration-related or reproducible mutagenic effects compared to solvent controls with and without S9 in any strain.

In the *Salmonella*/microsome test, using preincubation for 60 minutes at 37 °C, tebuconazole produced a slightly thin background lawn of non-revertant colonies, together with a reduction in revertant colony numbers at 5000 µg/plate. No test item precipitate was reported on the plates at any doses tested in either the presence or absence of metabolic activation. In agreement with the plate incorporation assay, there were no biologically relevant, concentration-related or reproducible mutagenic effects compared to solvent controls with and without S9 in any strain.

Conclusion
Overall, tebuconazole was not mutagenic in a guideline, modern *Salmonella typhimurium/Escherichia coli* reverse mutation assay (either the plate incorporation test or the pre-incubation experiment) up to cytotoxic/limit concentrations in the presence and absence of metabolic activation.

B.6.4.1.3. *In Vitro Mammalian Chromosomal Aberration Test (human lymphocytes)*

One *in vitro* chromosome aberration test is available. This was already considered in the original DAR (2006).

Previous evaluation:	In Tebuconazole DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.4.1.3/01
Study title	HWG 1608 - *In vitro* cytogenetic study with human lymphocytes for the detection of induced clastogenic effects
Test substance	(HWG 1608) Tebuconazole
Purity (%) **Batch no.**	96.5 1616001/86
Test system	Primary culture of human lymphocytes from the blood of one male and one female (healthy) donor
Groups	1000 cells per culture (4000 per concentration).

Concentration	Without S-9 mix: 0, 3, 10 and 30 µg/ml culture medium With S-9 mix: 0, 30, 100 and 300 µg/ml culture medium
Vehicle	DMSO
GLP	Yes
Guideline	OECD Guideline 473
Deviation	The following deviations from the OECD-Guideline 473 (2016) occurred: - With S-9 mix, only one test concentration met acceptability criteria (30) due to excessive cytotoxicity at 100 and 300. - Less than recommended number of metaphases per scored per concentration
Acceptable	Acceptable in a WoE approach. Despite the identified limitations, a modern *in vitro* micronucleus study is available.
Result	No clastogenic effect on human lymphocytes in concentrations up to 30 µg/ml without S-9 mix and up to 300 µg/ml with S-9 mix.

Methods
Human lymphocytes were gained from the blood of one male and one female healthy donor. There were two cultures per donor per concentration. The ratio of number of cells or metaphases evaluated was always 1:1 for cultures from male or female donor, respectively.

Human lymphocytes were stimulated to divide by the plant lectin, phytohaemagglutinin, in the culture medium and then were treated with the test compound. After addition of the spindle inhibitor colcemid, both the mitotic index and the chromosome aberration rate were determined.

The treatment concentrations selected were based on a pilot study in which the concentrations were 1, 10, 100, 1000 and 5000 µg/ml. The results indicated a higher cytotoxicity of test substance when applied without S-9 mix.

Positive controls used were:
Without S-9 mix: Mitomycin C (0.15 µg/ml)
With S-9 mix: Cyclophosphamide (15 µg/ml)

The mitotic index was determined by counting 1000 cells per culture (4000 per concentration). Approximately 100 metaphases per sex and test group were evaluated (200 per concentration). The structural chromosome damage was assessed by using the terminology defined by Rieger and Michaels (Die Chromosome-mutation, VEB Gustav Fischer Verlag, Jena, 1967).

Results
100 and 300 µg/ml of the test substance with S-9 mix showed cytotoxic effects on lymphocytes.

After treatment of lymphocytes at concentrations of up to 30 µg/ml without S-9 mix and 300 µg/ml with S-9 mix, respectively, tebuconazole produced a decrease in mitotic index in human lymphocyte cultures only with S-9 mix. Without S-9 mix no such effect was noted.

Evaluation of the individual groups with respect to parameters relevant for evaluating clastogenicity detected no variations of biological relevance between the groups.

Table 6.4-4. Summary results of the cytogenetic study without metabolic activation

µg/ml	**Evaluated metaphases**	**Metaphased with aberrations incl. gaps**		**Metaphased with aberrations excl. gaps**		**Metaphased with exchanges**		**Polyploid cells in *x* evaluated metaphases**	
		n	%	n	%	n	%	n *x*	%
DMSO 0	200	14	7.0	7	3.5	0	0	1 400	0.3
3	200	12	6.0	7	3.5	0	0	0 400	0
10	200	8	4.0	4	2.0	0	0	1 400	0.3
30	200	11	5.5	7	3.5	0	0	0 400	0
MMC 0.15	200	109*	54.5	67*	33.5	12*	6.0	1 400	0.3

* $p \leq 0.01$ in chi^2 test

Table 6.4-5. Summary results of the cytogenetic study with metabolic activation

µg/ml	Evaluated metaphases	Metaphased with aberrations incl. gaps		Metaphased with aberrations excl. gaps		Metaphased with exchanges		Polyploid cells in *x* evaluated metaphases		
		n	%	n	%	n	%	n	*x*	%
DMSO 0	200	22	11.0	12	6.0	0	0	0	400	0
30	200	24	12.0	8	4.0	0	0	0	400	0
100	200	14	7.0	4	2.0	0	0	0	300	0
300	Only cell fragments found									
CYCL 15	200	82*	41.0	53*	26.5	8*	4.0	0	400	0

* $p \leq 0.01$ in chi^2 test

The results for the positive controls mitomycin C and cyclophosphamide indicated a clear clastogenic effect and documented the system's sensitivity.

Conclusion
Under the stated test conditions, tebuconazole did not show a clastogenic effect on human lymphocytes in concentrations up to 30 µg/ml without S-9 mix and up to 300 µg/ml with S-9 mix (i.e. concentrations producing cytotoxicity).

B.6.4.1.4. *In vitro mammalian cell micronucleus test (human lymphocytes)*

A modern *in vitro* micronucleus test is available. This has been submitted by the BTF for the purposes of renewal.

Previous evaluation:	None – submitted for the purpose of renewal (study owned by Bayer Task force)

Study ID	B.6.4.1.4/01
Study title	Tebuconazole: Micronucleus test in human lymphocytes *in vitro*
Test substance	Tebuconazole
Purity (%) **Batch no.**	95.7 Specification no: 102000006666 Batch no: 2015-005886
Test system	Human peripheral blood lymphocytes
Groups	1000 binucleated cells / culture. 2 parallel cultures / dose
Concentration	Experiment: I; Exposure period: 4 hrs; S9 mix: − ; Concentrations in µg/mL: 13.0, 22.7, **39.8**, **69.6**, **122**, 213, 373[P], 653[P], 1143[P], 2000[P] Experiment: IIA; Exposure period: 20 hrs; S9 mix: − ; Concentrations in µg/mL: 6.5, 9.8, 14.6, **21.9**, **32.9**, **49.4**, 74.1, 111, 167, 250 Experiment: IIB; Exposure period: 20 hrs; S9 mix: − ; Concentrations in µg/mL: 17.7, **35.3**, 40.6, **46.7**, 53.7, **61.8**, 71.1, 81.7 94.0 Experiment: I; Exposure period: 4 hrs; S9 mix: + ; Concentrations in µg/mL: 13.0, 22.7, **39.8**, **69.6**, **122**, 213, 373[P], 653[P], 1143[P], 2000[P] Experiment: IIB; Exposure period: 4 hrs; S9 mix: + ; Concentrations in µg/mL: **81.7**, **94.0**, **108[P]**, 124[P], 143[P], 164[P], 189[P], 217[P], 250[P]
Vehicle	DMSO
GLP	Yes
Guideline	OECD guideline 487
Deviation	The following deviations from the OECD-Guideline 487 (2016) occurred: - recovery phase and harvest time for the treatment were slightly modified compared to the guideline proposal. This was done based on non-GLP validation experiments performed to get distinct and statistically significant responses in positive controls.
Acceptable	Acceptable
Result	Tebuconazole did not induce micronuclei as determined by the *in vitro* micronucleus test in human lymphocytes and is considered to be non-mutagenic in this *in vitro* micronucleus test,

	when tested up to cytotoxic or precipitating concentrations.

Methods
Tebuconazole (dissolved in DMSO) was tested *in vitro* for its potential to induce micronuclei in Human peripheral blood lymphocytes in the absence and presence of metabolic activation (rat liver-derived S9 mix) in three independent experiments (I, IIA, and IIB). In Experiment I, the exposure period was 4 hours, with and without S9 mix, with concentrations of 13 - 2000 µg/mL. Experiment IIB included another 4 hour exposure period with S9 mix at concentrations 81.7 – 250 µg/mL. In the experiments IIA and IIB, the exposure period was extended to 20 hours without S9 mix at concentrations 6.5-250 µg/mL (IIA) and 17.7-250 µg/mL (IIB) respectively. Concentrations were selected on the basis of a preliminary cytotoxicity test (13-2000 µg/mL) and the requirements of OECD Guideline 487. Slides were prepared from harvested cells and at least 2000 cells per test group (two parallel cultures of 1000 cells per culture) were scored for the number of micronuclei-containing binucleated cells. To determine a cytotoxic effect the CBPI was determined in 500 cells per culture and cytotoxicity is described as % cytostasis.

Results
Cytotoxic effects
Without S9, cytotoxic effects occurred at 213 µg/mL and above after 4 hours treatment and at 74.1 µg/mL and above after 20 hours treatment. With S9, cytotoxic effects were observed at 164µg/mL and above.

Precipitation
Without S9, precipitation in the medium occurred at 373 µg/mL and above after 4 hours treatment and did not occur after 20 hours treatment. With S9, precipitation was observed at 108 µg/mL and above.

Table 6.4-6. Summary of concentrations inducing cytotoxicity and precipitation in experiment ± S9

	- S9			**+ S9**	
	4 hours (I)	**20 hours (IIA)**	**20 hours (IIB)**	**4 hours (I)**	**4 hours (IIB)**
Precipitation	373	n/a	n/a	373	108
Cytotoxicity	213	74.1	n/A	213	164

No relevant influence on osmolarity or pH was observed.

Concentrations chosen for micronucleus assessment
Concentrations of 39.8 - 122 µg/mL (with and without S9 mix, 4 hours treatment) were chosen for micronucleus assessment in experiment I. Higher concentrations were excluded due to excessive cytotoxicity. In experiments IIA and IIB concentrations 21.9 – 49.4 µg/mL and 35.5 – 61.8 µg/mL were chosen respectively (without S9 mix, 20 hours treatment), whilst and 81.7 – 108 µg/mL (with S9 mix, 4 hours treatment) were chosen for micronucleus assessment in experiment IIB.

Cytogenetic results
No concentration dependent increase in cells with micronuclei compared to the solvent (DMSO) control was seen with and without metabolic activation after treatment for 4 hours, or without metabolic activation after treatment for 20 hours.

Solvent and positive controls revealed the expected results of micronucleated cells demonstrated the suitability and sensitivity of the test system, thereby ensuring the validity of the test.

Table 6.4-7. Summary of cytogenetic results

Exp.	Preparation interval	Test item concentration in µg/mL	Proliferation index CBPI	Cytostasis in %*	Micronucleated cells in %**	95% Ctrl Limit Micronucleated cells in % (2014-2015)
Exposure period 4 hours			without S9 mix			
I	40 hrs	Solvent control[1]	1.99		0.15	0.07 – 1.15
		Positive control[2]	1.77	22.5	13.20 S	1.48 – 21.85
		39.8	1.96	2.9	0.35	
		69.6	1.97	1.6	0.20	

Exp.	Preparation interval	Test item concentration in µg/mL	Proliferation index CBPI	Cytostasis in %*	Micronucleated cells in %**	95% Ctrl Limit Micronucleated cells in % (2014-2015)
		122	1.78	20.6	0.30	
with S9 mix						
I	40 hrs	Solvent control[1]	2.04		0.25	0.08 – 1.20
		Positive control[3#]	1.79	24.6	3.38 S	0.88 – 8.73
		39.8	1.86	17.7	0.60	
		69.6	1.82	20.9	0.10	
		122	1.78	25.0	0.35	
IIB	40 hrs	Solvent control[1]	2.10		0.45	0.08 – 1.20
		Positive control[3]	1.82	25.8	9.65 S	0.88 – 8.73
		81.7	1.91	17.7	0.45	
		94.0	1.85	22.8	0.25	
		108[P]	1.79	28.6	0.40	
Exposure period 20 hours			without S9 mix			
IIA	40 hrs	Solvent control[1]	1.75		0.95	0.05 – 1.05
		Positive control[4]	1.37	50.6	4.45 S	1.69 – 5.41
		21.9	1.63	15.8	0.65	
		32.9	1.53	29.3	0.30	
		49.4	1.50	33.0	0.25	
IIB	40 hrs	Solvent control[1]	1.88		0.50	0.05 – 1.05
		Positive control[5]	1.55	37.8	2.70 S	1.69 – 5.41
		35.3	1.71	19.2	0.05	
		46.7	1.63	28.3	0.15	
		61.8	1.41	53.7	0.10	

* For the positive control groups and the test item treatment groups the values are related to the solvent controls
** The number of micronucleated cells was determined in a sample of 2000 binucleated cells
\# The number of micronucleated cells was determined in a sample of 4000 binucleated cells
S The number of micronucleated cells is statistically significantly higher than corresponding control values
P Precipitation occurred at the end of treatment
1 DMSO 0.5 % (v/v)
2 MMC 1.0 µg/mL
3 CPA 15.0 µg/mL
4 Demecolcin 125 ng/mL
5 Demecolcin 75 ng/mL

Conclusion
Based on the results of this guideline, modern study, tebuconazole is not considered to have a chromosome-damaging (clastogenic) effect or to induce numerical chromosomal aberrations (aneugenic activity) leading to micronucleus formation under *in vitro* conditions in human lymphocytes in the absence and presence of metabolic activation, up to concentrations causing cytotoxicity. Therefore, tebuconazole is considered to be non-mutagenic in this *in vitro* micronucleus test, when tested up to cytotoxic or precipitating concentrations.

B.6.4.1.5. *In vitro mammalian cell gene mutation assay in CHO-cells (HPRT-test)*

Two *in vitro* mammalian cell gene mutation assays are available. One was considered in the original DAR (2006) and one has been submitted by the BTF for the purposes of renewal.

1)

Previous evaluation:	In Tebuconazole DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.4.1.5/01
Study title	HWG 1608 - Mutagenicity study for the detection of induced forward mutations in the CHO-HGPRT assay *in vitro*

Test substance	(HWG 1608) Tebuconazole
Purity (%) **Batch no.**	96.6 1616001/86
Test system	Chinese hamster Ovary (CHO) cells
Groups	Three trials were performed for each treatment
Concentration	Without S-9 mix: 80, 90, 92.5, 95, 97.5 and 100 µg/ml culture medium. With S-9 mix (5%): 12.5,25, 50, 100, 150 and 200 µg/ml culture medium.
Vehicle	DMSO
GLP	Yes
Guideline	OECD Guideline 476
Deviation	This is an old study and deviations from the OECD-Guideline 476 (2016) occurred; however, a new fully guideline compliant study (Sokolowski, A.; 2017) is available.
Acceptable	Acceptable in a WoE approach. Despite the identified limitations, a second modern assay has been submitted.
Result	Not considered mutagenic in the CHO-HPRT assay in concentrations up to 100 µg/ml without S-9 mix or up to 150 µg/ml with S-9 mix.

Methods
Although not stated in the study report the study was done according to OECD guideline 476. Three trials were performed for each treatment with and without activation. In the first two trials, no duplicates were used. The third trial was performed employing duplicates.

The test concentrations were based on a pilot study in which the doses ranged from 5 to 125 µg/ml without S-9 mix and from 3.9 to 1000 µg/ml with S-9 mix.

Positive controls:
Without S-9 mix: Ethylmethanesulfonate (907 µg/ml)
With S-9 mix: 3-Methylcholanthrene (5 µg/ml)

Results
Under both treatment conditions (with and without S-9 mix), the test substance induced cytotoxic effects. Without S-9 mix, a concentration-related decrease in relative population growth was observed only in the third trial over the whole treatment range. With S-9 mix, all cells were lost at a concentration of 200 µg/ml in all three trials. In addition, cells were also killed in the third trial at 150 µg/ml. In all assays, high toxicities were induced so that the treated cultures showed concentration-related decreases in both relative survival to treatment and relative population growth (Tables 6.4-8. and 6.4-9.).

There was neither concentration related nor reproducible increases in mutant frequency with the test substance. In contrast, the positive controls revealed a clear mutagenic effect in this assay.

Table 6.4-8. Gene Mutation Assay

Treatment without S-9 mix				
Concentration [µg/ml]	**Mutant Frequency (Thioguanin-resistant mutants per 10^6 clonable cells)**			
	1st trial	2nd trial	3rd trial with duplicates	
Negative control	11.4	17.4	0.7	2.4
Vehicle control	20.1	13.7	0.8	3.4
80	30.7	10.1	1.7	3.1
90	14.9	9.9	2.6	0.8
92.5	25.7	9.4	2.2	0.9
95	22.0	10.7	1.4	2.4
97.5	25.4	10.9	5.5	3.8
100	7.4	1.7	2.8	3.7
Positive control	67.8*	106.0*	144.5*	171.5*

* significant increase, $p<0.05$

Table 6.4-9. Gene Mutation Assay

Treatment with 5% S-9 mix					
Concentration [µg/ml]	**Mutant Frequency (Thioguanin-resistant mutants per 10^6 clonable cells)**				**Comments**
	1st trial	2nd trial	3rd trial with duplicates		
Negative control	16.4	11.1	1.2	2.8	
Vehicle control	19.8	18.1	1.2	2.3	
12.5		29	2.8	6.5	in 1st trial no colonies were available
25	29.3	20	3.6	2.3	
50	9	21.6	2.6	3.7	
100	27.9	15.5	9.4	1.3	
150	23.5	26.1			in 3rd trial all cells were lost due to cytotoxicity
200					1st trial: precipitation of test article 2nd and 3rd trial: all cells were lost due to cytotoxicity
Positive control	59.5*	191.5*	32.5*	41.4*	

* significant increase, $p<0.05$

Conclusion
Under the stated test conditions, tebuconazole was not considered mutagenic in the CHO-HPRT assay in concentrations up to 100 µg/ml without S-9 mix or up to 150 µg/ml with S-9 mix (i.e. concentrations producing cytotoxicity).

2)

Previous evaluation:	None – submitted for the purpose of renewal (study owned by Bayer Task force)

Study ID	B.6.4.1.5/02
Study title	Tebuconazole: Mammalian cell gene mutation assay in Chinese hamster 79 cells *in vitro* (HPRT-Locus)
Test substance	Tebuconazole
Purity (%) **Batch no.**	95.7 2015-005886,
Test system	V79 cell cultures
Groups	three independent experiments, using two parallel cultures each.
Concentration	Pre-test for cytotoxicity: 0, 15.6, 31.3, 62.5, 125, 250, 500, 1000, 2000 µg/mL (±S9) Experiment I: 0, 3.9, 7.8, 15.7, 31.3, 46.9, 62.5, 125, 250 µg/mL (±S9) Experiment II: 0, 7.5, 15, 30, 60, 70, 80, 90, 100, 120 µg/mL (-S9) Experiment III: 0, 7.5, 15, 30, 60, 70, 80, 90, 100, 120 µg/mL (+S9)
Vehicle	DMSO
GLP	Yes
Guideline	OECD Guideline 476
Deviation	The following deviations from the OECD-Guideline 476 (2016) occurred: None
Acceptable	Acceptable
Result	No significant and reproducible test substance induced increases in mutant frequencies were observed with and without metabolic activation up to cytotoxic concentrations. Based on these results, tebuconazole is considered to be non-mutagenic in the V79/HPRT Forward Mutation Assay, both with and without metabolic activation.

Methods
Tebuconazole was evaluated for point mutagenic effects at the hypoxanthine-guanine phosphoribosyl transferase locus (HPRT forward mutation assay) in Chinese hamster V79 cell cultures. The study was performed in three independent experiments, using two parallel cultures each. Based on preliminary cytotoxicity testing, cells were treated with concentrations ranging from 3.9 to 250 ug/ml, in the presence or absence of metabolic activation (S9

mix) for 4 hours. The results of experiment II with metabolic activation are not reported. The data of the repeat experiment are reported as Experiment III. The study design included the testing of appropriate positive and negative controls.

Results

Pre-test for cytotoxicity

A relevant cytotoxic effect indicated by a relative cloning efficiency of 50 % or below occurred at 31.3 µg/mL in the presence of metabolic activation. At higher concentrations the cell growth was completely inhibited. In the absence of metabolic activation, a severe cytotoxic effect occurred at 62.5 µg/mL. At the next higher concentrations the cell growth was completely inhibited. Precipitation occurred at 250.0 µg/mL and above after 4 hours treatment with and without metabolic activation. The concentration range of Experiment I was set according to data generated in the pre-experiment. The concentration range of Experiment II and III was based on the data generated in the first main experiment.

Gene mutation assays

In experiment I, excessive cytotoxicity was observed at 125 and 250 µg/mL and precipitation of tebuconazole in the culture medium was observed at 250 µg/mL. Without S9, tebuconazole induced no biologically-relevant decreases in survival or in relative population growth. There were also no biologically-relevant or statistically significant increases in mutant frequencies in the absence of S9.

With S9, cloning efficiency was 49.5 % at 62.5 µg/m, consistent with the cytotoxicity seen at this concentration in the pre-experiment. No other biologically-relevant decreases in survival or in relative population growth were seen with S9. At 62.5 µg/mL, a statistically significant increase in mutant frequencies was seen; the upper border of the 95 % confidence interval (29.4 mutants per 10^6 cells) was slightly exceeded, however, the value of this concentration (31.0 mutants per 10^6 cells) was still clearly within the historical solvent control data range (2.4 to 39.2 mutants per 10^6 cells) and, thus, the observation has to be regarded as biologically irrelevant. Furthermore this minor increase was not reproduced in the experiment III. No biologically relevant or statistically significant increases in mutant frequencies in the presence of S9 at other tested concentrations. Tebuconazole was therefore non-mutagenic in the activation and non-activation assay in experiment I.

Table 6.4-10. Summary of mutant frequency following treatment with tebuconazole in the presence or absence of metabolic activation in experiment I

Concentration (µg/mL)	**S9 mix**	**Relative CE I (% control)**	**Relative cell density (% control)**	**Relative adjusted CE I ± SD (%)**	**Mutant frequency (x 10^{-6} cells)**	**95% confidence interval**
0$	-	100.0	100.0	100.0	18.6	1.7 – 30.2
3.9		culture was not continued #				
7.8		80.6	90.0	71.9	21.5	1.7 – 30.2
15.7		89.7	88.9	79.2	22.2	1.7 – 30.2
31.3		88.5	89.8	79.5	17.7	1.7 – 30.2
46.9		95.5	86.1	81.8	29.0	1.7 – 30.2
62.5		74.2	70.5	52.4	17.9	1.7 – 30.2
125.0		##	3.0	culture was not continued ##		
250.0		##P	1.8P	culture was not continued ##		
EMS 300		94.4	91.3	86.3	263.7	
0$	+	100.0	100.0	100.0	19.6	2.0-29.4
3.9		culture was not continued #				
7.8		107.2	80.1	85.9	16.5	2.0-29.4
15.7		111.0	72.5	80.2	25.9	2.0-29.4
31.3		100.4	80.2	80.6	24.7	2.0-29.4
46.9		99.1	76.3	75.9	29.2	2.0-29.4
62.5		49.5	74.4	34.9	31.0*t	2.0-29.4
125.0		##	5.5	culture was not continued ##		
250.0		##P	1.9P	culture was not continued ##		
DMBA 2.3		103.0	78.3	80.7	190.8	

*: $p < 0.05$ (t-test) t: $p < 0.05$ (trend test)
CE: Cloning efficiency P: precipitation $: solvent control

Concentration (µg/mL)	S9 mix	Relative CE I (% control)	Relative cell density (% control)	Relative adjusted CE I ± SD (%)	Mutant frequency (x 10^{-6} cells)	95% confidence interval

[a]: The 95% confidence interval is derived from the mean value plus/minus 2 times the standard deviation.
culture was not continued as only 4 analysable concentrations are requested by the guideline
culture was not continued due to exceedingly severe cytotoxic effects

The experimental part with S9 mix in Experiment II was judged as invalid, since the mean mutant frequency of solvent control was outside the 95 % confidence interval of the laboratory's historical solvent control data and, thus, did not fulfil the requirements of the current OECD Guideline 476. Therefore, the results of experiment II with metabolic activation are not reported. The data of the repeat experiment are reported as Experiment III.

In experiment II a cytotoxic effect, indicated by an adjusted cloning efficiency I below 50%, was observed at 70.0 µg/mL (40.9 %) and above without metabolic activation. In experiment III a cytotoxic effect was observed at 60.0 µg/mL (35.9 %) and above with metabolic activation. Concentrations above these cytotoxic concentrations were therefore not scored for gene mutations.

In experiment II without metabolic activation the upper border of the 95 % confidence interval (30.2 mutants per 10^6 cells) was slightly exceeded at 15.0 µg/mL (32.3 mutants per 10^6 cells) and at 80.0 µg/mL (31.6 mutants per 10^6 cells), respectively. The values of both concentrations were still clearly within the historical solvent control data range (3.4 to 41.0 mutants per 10^6 cells), and no concentration dependency was observed. Therefore, it has to be concluded that the single statistically significant response (at 15 µg/mL) is biologically irrelevant.

With and without S9, tebuconazole induced no biologically relevant decreases in survival or in relative population growth. There were also no biologically relevant or statistically significant increases in mutant frequencies in the presence or absence of S9. Tebuconazole was therefore non-mutagenic in the activation and non-activation assay in repeat experiment (II with S9 and III with S9).

Table 6.4-11. Summary of mutant frequency following treatment with tebuconazole in the presence or absence of metabolic activation in experiment II and III

Concentration (µg/mL)	S9 mix	Relative CE I (% control)	Relative cell density (% control)	Relative adjusted CE I ± SD (%)	Mutant frequency (x 10^{-6} cells)	95% confidence interval
Experiment II						
0$	-	100.0	100.0	100.0		1.7 – 30.2
7.5		93.1	106.0	98.6	#	
15.0		86.2	96.6	83.2	32.3*	1.7 – 30.2
30.0		93.5	79.1	73.9	26.4	1.7 – 30.2
60.0		75.7	83.1	62.9	19.2	1.7 – 30.2
70.0		40.9	71.0	31.1	23.1	1.7 – 30.2
80.0		9.1	35.5	3.3	31.6	1.7 – 30.2
90.0		culture was not continued ##				
100.0		culture was not continued ##				
120.0		culture was not continued ##				
EMS 300		93.1	107.1	99.7	386.5	
Experiment III						
0$	+	100.0	100.0	100.0		2.0-29.4
7.5		93.2	87.3	81.3	22.0	2.0-29.4
15.0		89.2	84.4	75.1	23.0	2.0-29.4
30.0		54.3	97.3	52.8	28.5	2.0-29.4
60.0		35.9	78.9	28.6	13.7	2.0-29.4
70.0		6.7	44.0	3.3	##	2.0-29.4
80.0		8.9	11.2	1.0	##	2.0-29.4
90.0		##	6.6	culture was not continued ##		
100.0		culture was not continued ##				

Concentration (µg/mL)	S9 mix	Relative CE I (% control)	Relative cell density (% control)	Relative adjusted CE I ± SD (%)	Mutant frequency (x 10^{-6} cells)	95% confidence interval
120.0		culture was not continued ##				
DMBA 2.3		91.3	102.0	93.4	105.2	

*: $p < 0.05$ (t-test) t: $p < 0.05$ (trend test)
CE: Cloning efficiency P: precipitation $: solvent control
[a]: The 95% confidence interval is derived from the mean value plus/minus 2 times the standard deviation.
culture was not continued as only 4 analysable concentrations are requested by the guideline
culture was not continued due to exceedingly severe cytotoxic effects

The negative controls were within the normal range. The positive control substances induced clear mutagenic effects and demonstrated the sensitivity of the test system and the activity of the S9 mix.

Table 6.4-12. Historical control data (studies performed 2014-2016)

Number of mutant colonies per 10^6 cells		
without metabolic activation (4 hours treatment time)		
	Positive control EMS 150 and 300 µg/mL	**Solvent control (medium, acetone, water, DMSO, ethanol, THF, EGDE)**
Range:	53.9 – 872.3	3.4 – 41.0
Mean value:	190.3	15.9
Standard deviation:	88.4	7.1
95% confidence interval:	--	1.7 – 30.2
Number of studies:	111	111
with metabolic activation (4 hours treatment time)		
	Positive control DMBA 1.1 and 2.3 µg/mL	**Solvent control (medium, acetone, water, DMSO, ethanol, THF, EGDE)**
Range:	56.7 – 739.9	2.4 – 39.2
Mean value:	215.8	15.7
Standard deviation:	110.9	6.8
95% confidence interval	--	2.0 – 29.4
Number of studies:	105	105

Conclusion
Overall, no significant and reproducible test substance induced increases in mutant frequencies were observed with and without metabolic activation up to cytotoxic concentrations. Based on these results, tebuconazole is considered to be non-mutagenic in the V79/HPRT Forward Mutation Assay, both with and without metabolic activation.

B.6.4.1.6. *In vitro sister chromatid exchange assay in mammalian cells (CHO cells)*

A non-standard *in vitro* SCE assay is available. This was considered in the original DAR (2006).

Previous evaluation:	In Tebuconazole DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.4.1.6/01
Study title	HWG 1608 - Sister chromatid exchange assay in Chinese hamster ovary (CHO) cells
Test substance	(HWG 1608) Tebuconazole
Purity (%) **Batch no.**	96.5 1616001/86

Test system	Chinese hamster ovary cells – CHO-K1 cell line
Concentration	0, 4, 8, 15, and 30 µg/mL without metabolic activation; 0, 15, 30, 60, and 120 µg/mL with metabolic activation
Vehicle	DMSO
GLP	Yes, except the analytical controls of the test substances were not performed by the testing laboratory.
Guideline	OECD guideline 479
Deviation	The following deviations from the OECD-Guideline 479 (1986) occurred: none
Acceptable	Acceptable – as supplementary, as non-standard test
Result	Tebuconazole was negative in the Sister Chromatid Exchange assay in concentrations up to 30 µg/mL in the absence of S-9 mix and up to 120 µg/mL in the presence of metabolic activation

Methods
Tebuconazole was tested in the sister chromatid exchange assay *in vitro*, using Chinese hamster ovary cells. Solvent was dimethyl sulphoxide (DMSO). Triethylenemelamine (2.5 µg/mL) and cyclophosphamide (0.25 µg/mL) were used as positive controls. To achieve metabolic activation the CHO cells were incubated for 2 hours with S-9 mix after which a rinsing of the cells took place and were supplied with complete growth medium. The exposure period at 37ºC was 30 hours for cells with and without metabolic activation. Metaphase cells were harvested 2 hours after addition of Colcemid.

Results
There were no significant increases in the frequency of sister chromatid exchanges observed at any concentration levels with or without metabolic activation. Due to cytotoxicity there were no metaphase cells to evaluate in any of 120 µg/ml flasks.

Conclusion
Tebuconazole was negative in the Sister Chromatid Exchange assay in concentrations up to 30 µg/mL in the absence of S-9 mix and up to 120 µg/mL in the presence of metabolic activation when tested in Chinese Hamster Ovary cells.

B.6.4.1.7. *In vitro DNA Damage and Repair, Unscheduled DNA Synthesis assay in Mammalian Cells*

An *in vitro* UDS assay is available. This was considered in the original DAR (2006).

Previous evaluation:	In Tebuconazole DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.4.1.7/01
Study title	HWG 1608 techn. - Mutagenicity test in the rat primary hepatocyte unscheduled DNA synthesis assay
Test substance	(HWG 1608) Tebuconazole
Purity (%) **Batch no.**	96.5 1616001/86
Test system	Freshly prepared hepatocytes from an adult male Fischer 344 rat.
Concentration	0.504, 1.01, 2.52, 5.04, 10.1, and 25.2 µg/mL
Vehicle	DMSO
GLP	Yes
Guideline	OECD guideline 482
Deviation	The following deviations from the OECD-Guideline 482 (1986) occurred: -none
Acceptable	Acceptable – supplementary as non-standard test
Result	Not mutagenic in the primary rat hepatocyte unscheduled DNA synthesis assay in the dose range 0.504 ¬ 25.2 µg/mL.

Methods
Tebuconazole was tested for mutagenic effects in the *in vitro* rat primary hepatocyte unscheduled DNA synthesis (UDS) assay. The selection of the concentration range was a result of an initial testing of the cytotoxicity of the test substance with concentrations from 0.025 to 1000 µg/mL. The freshly prepared rat hepatocytes were exposed for 18 hours to tebuconazole dissolved in DMSO. The positive control was 2-acetyl aminofluorene: 0.1 µg/mL. The test was performed in triplicate. The number of labelled grains per nucleus was determined by autoradiography.

Results
Tebuconazole did not induce significant changes in the nuclear labelling of primary rat hepatocytes for the applied concentration range whereas the positive control induced large increases in nuclear labelling and thus demonstrated the sensitivity of the assay. A good range of toxicities was induced (104.1 % – 55.8 % survival).

Conclusion
Tebuconazole was not mutagenic in the primary rat hepatocyte unscheduled DNA synthesis assay in the concentration range 0.504 – 25.2 µg/mL.

B.6.4.1.8. ***Rec-assay with spores in a bacterial system***

A non-standard *in vitro* rec-assay is available. This was considered in the original DAR (2006).

Previous evaluation:	In Tebuconazole DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.4.1.8/01
Study title	HWG 1608 – Rec-assay with spores in the bacterial system
Test substance	(HWG 1608) Tebuconazole
Purity (%) **Batch no.**	98.0 816096181
Test system	Spores of Bacillus subtilis strains H17 (Rec+) and M45 (Rec-)
Concentration	0.313, 0.625, 1.25, 2.5, 5, 10, and 20 µg/plate (both with and without metabolic activation)
Vehicle	DMSO
GLP	Yes
Guideline	Japanese MAFF (59 Nohsan No. 4200). No OECD equivalent
Deviation	n/a
Acceptable	Acceptable – supplementary as non-standard test
Result	No DNA-damaging effects, with and without metabolic activation, in this rec-assay test in spores of two strains of B. subtilis in the dose range 0.313 to 20 µg/plate.

Methods
Tebuconazole, dissolved in DMSO, was investigated in the Rec-assay with spores for DNA-damaging effects.

Positive controls:
mitomycin C (– S-9 mix): 0.005 – 0.01 µg/plate
2-aminoanthracene(+/– S-9 mix): 5.0 – 20.0 µg/plate
Negative control:
Kanamycin sulphate (– S-9 mix): 0.5 – 1.0 µg/plate

Results
Slight growth inhibition was observed for both strains at the highest dose of 20 µg/plate (indicating bacteriotoxicity at this concentration) with and without metabolic activation, but no difference in growth was detected between the two strains at lower concentrations.

The positive controls mitomycin C and 2-aminoanthracene showed a marked growth inhibition in *B. subtilis* strain M45, indicating that the test system is a proper system for detecting DNA-damaging properties. The negative control substance induced slight growth inhibition in both strains (difference below 5 mm) therefore the DNA-

damaging activity of that substance was negative.

Conclusion
Tebuconazole demonstrated no DNA-damaging effects, with and without metabolic activation, in this rec-assay test in spores of two strains of *B. subtilis* in the dose range 0.313 to 20 µg/plate.

B.6.4.2. *In vivo* studies in somatic cells

B.6.4.2.1. *Mouse micronucleus test*

Four *in vivo* micronucleus studies are available : two were considered in the original DAR (2006) and two (one by the BTF and one by the OTF) were submitted for the purposes of renewal.
1)

Previous evaluation:	In Tebuconazole DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.4.2.1/01
Study title	HWG 1608 – Micronucleus test on the mouse to evaluate for mutagenic effect
Test substance	(HWG 1608) Tebuconazole
Purity (%) **Batch no.**	95.3 16007/83
Test animals	NMRI mice
Groups	5/sex/dose group
Doses	200, 2000 mg/kg bw
Route	Oral, gavage
Vehicle	1.0% Cremophor emulsion
GLP	Yes
Guideline	OECD guideline 474
Deviation	The following deviations from the OECD-Guideline 474 (2016) occurred: - Only 1000 polychromatic erythrocytes scored for micronuclei - No justification for single treatment - The reporting was different from the requirements in the guideline, but this does not affect the scientific outcome of the study (reported prior to guideline).
Acceptable	Acceptable – supplementary, as a new, modern study is available.
Result	Not mutagenic in the somatic *in vivo* mouse micronucleus test when administered in single oral doses (200 to 2000 mg/kg bw) to male and female NMRI mice but was found to inhibit erythropoiesis.

Methods
Tebuconazole, suspended in 1.0 % Cremophor emulsion, was administered in a single dose by gavage to 5 male and 5 female NMRI mice per dose and exposure time group, including negative and positive controls in accordance with the table below. The administered volume was 10 mL/kg bw. Doses of 200, 500 and 2000 mg/kg bw were tested.

Positive control:
Cyclophosphamide 20.0 mg/kg bw.

After the exposure (preparation) time the animals were sacrificed by decapitation and the femoral marrow was prepared.

Table 6.4-13. Dosing schedule and preparation time

	Dose [mg/kg bw]	**Preparation time [hours]**
Test 1		

Negative control	0	24
Tebuconazole	2000	24
Tebuconazole	2000	48
Tebuconazole	2000	72
Positive control	20*	24
Test 2		
Negative control	0	24
Tebuconazole	500	24
Tebuconazole	500	48
Tebuconazole	500	72
Positive control	20*	24
Test 3		
Negative control	0	48
Tebuconazole	200	48
Positive control	20*	24

* corresponding to 29 mg/kg bw of Endoxan

One thousand polychromatic erythrocytes were counted per animal and the incidence of cells with micronuclei was established.

The ratio of polychromatic to normochromatic erythrocytes was also noted to detect possible pathological bone marrow to be excluded from the examination and to gain knowledge of potential general effects on erythropoiesis.

Results
The animals did not show any clinical signs after a single oral application of doses up to and including 2000 mg/kg bw.

No indication of a mutagenic effect – no increase in micronucleated cells – was found after treatment with doses up to and including 2000 mg/kg bw but the formation of erythrocytes was affected from 200 mg/kg bw onwards.

The positive control had a clear mutagenic effect whereas an inhibition of erythropoiesis was not noted.

Table 6.4-14. Results of the cytogenetic test (group means)

Sampling time	**Dose (mg/kg)**	**Vehicle**	**Tebuconazole**			**Cyclophosphamide**
		0	**200[c]**	**500[b]**	**2000[a]**	**20**
24 h	Number of PE scored	1000	-	1000	1000	1000
	MPE/ 1000 PE	2.2[b] / 1.1[a]	-	2.5	1.9	14.4[c] / 13.7[b] / 12.1[a]
	PE/NE ratio[#1]	1.57[b] / 1.37 [a]	-	0.48	0.49	1.65[c] / 1.96[b] / 1.30[a]
48 h	Number of PE scored	1000[c]	1000	57	69	-
	MPE/ 1000 PE	2.5[c]	1.5	nr	nr	-
	PE/NE ratio[#1]	2.05[c]	0.61	0.28[#2]	0.13[#2]	-
72 h	Number of PE scored	-	-	1000	154	-
	MPE/ 1000 PE	-	-	1.8	nr	-
	PE/NE ratio[#1]	-	-	0.26	0.31[#2]	-

MPE: Micronucleated Polychromatic Erythrocytes
PE: Polychromatic Erythrocytes NE: Normochromatic Erythrocytes
nr: not relevant
[#1]: per 1000 counted cells [#2]: calculated from extrapolated values
[a]: Test 1 [b]: Test 2
[c]: Test 3

Conclusion
Tebuconazole was not mutagenic in the somatic *in vivo* mouse micronucleus test performed in accordance with OECD guideline 474 when administered in single oral doses from 200 to 2000 mg/kg bw to male and female NMRI mice, which caused cytotoxicity to the bone marrow, and hence providing evidence of target organ

exposure.

2)

Previous evaluation:	In Tebuconazole DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.4.2.1/02
Study title	HT 308 technical - Bone marrow micronucleus test by oral route in mice
Test substance	Tebuconazole technical
Purity (%) **Batch no.**	99.5 015/98
Test animals	Male and female mice. Crl:CD-1 (ICR) BR
Groups	5/sex/dose group
Doses	0 - 187.5 – 375 – 750 mg/kg/day total doses: 0 - 375 – 750 – 1500 mg/kg
Route	Oral
Vehicle	0.5 % aqueous methylcellulose solution
GLP	Yes
Guideline	OECD guideline 474
Deviation	The following deviations from the OECD-Guideline 474 (2016) occurred: - Only 2000 polychromatic erythrocytes scored for micronuclei
Acceptable	Acceptable - supplementary, as a new, modern study is available.
Result	Tebuconazole does not induce damage to the chromosomes or the mitotic apparatus of mice bone marrow cells after two oral administrations, with a 24-hour interval, at the dose-levels of 187.5, 375 or 750 mg/kg/day.

Methods
A range finding study was first conducted, in which male and female Swiss Ico:OFI mice (3 mice/sex/group) were orally administered 500 – 2000 mg/kg tebuconazole. Based on the findings in this range finding study, tebuconazole was administered in two doses of 187.5, 375 or 750 mg/kg/day (total doses: 0 - 375 – 750 – 1500 mg/kg) in a volume of 10 mL/kg to Crl:CD-1 (ICR) BR mice in the main test. The vehicle served as the negative, and CPA as the positive control. The animals were sacrificed 24 h after the administration of the last treatment. For each animal, 2000 polychromatic erythrocytes were evaluated for micronuclei. The polychromatic (PE) and normochromatic (NE) erythrocyte ratio was established by scoring a total of 1000 erythrocytes (PE+NE).

Results
Clinical signs and mortality
No clinical signs and no mortality were observed in the animals of both sexes given 375 or 187.5 mg/kg/day. At 750 mg/kg some clinical signs as hypoactivity, tremors or piloerection were noted.

Administration of tebuconazole did not lead to any biologically relevant increase in the number of polychromatic erythrocytes that contained micronuclei. The rate of micronuclei was close to the concurrent negative controls with no statistically significant differences observed.

The formation of erythrocytes was affected at all doses tested in females, and from 375 mg/kg day onwards in males (indicated by reduced PE/NE ratio). This inhibition of erythropoiesis indicated bone marrow toxicity and therefore exposure of the bone marrow to tebuconazole and/or its metabolites.

Table 6.4-15. Induction of nuclei in bone marrow cells (means ± SD; 24 h after last treatment)

Dose (mg/kg day)	Vehicle 0	Tebuconazole 187.5	 375	 750	Cyclophosphamide 50 mg/kg
males					
MPE/ 1000 PE	1.0 ± 1.1	0.4 ± 0.7	0.7 ± 0.6	0.6 ± 0.5	39.4*** ± 7.1
PE/NE ratio	0.8 ± 0.4	0.4 ± 0.3	0.2* ± 0.2	0.2* ± 0.1	1.0 ± 0.4
females					
MPE/ 1000 PE	0.9 ± 0.7	0.8 ± 0.8	0.7 ± 0.8	0.8 ± 0.8	27.2*** ± 7.3

PE/NE ratio	1.2 ± 0.4	0.4** ± 0.1	0.3** ± 0.2	0.1*** ± 0.1	0.3** ± 0.1

MPE: Micronucleated Polychromatic Erythrocytes
PE: Polychromatic Erythrocytes
NE: Normochromatic Erythrocytes
* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$

In the two vehicle control groups, the frequency of MPE was consistent with the historical data. The positive control for clastogenicity, CPA, led to the expected highly significant increase ($p < 0.001$) in the rate of polychromatic erythrocytes that contained micronuclei. The sensitivity and validity of the test system under the experimental conditions was therefore confirmed

Conclusion
Tebuconazole was not mutagenic in the somatic *in vivo* mouse micronucleus test performed in accordance with OECD guideline 474 when administered in two oral doses, with a 24-hour interval, from 187.5 to 750 mg/kg bw to male and female Crl:CD-1 mice, at which bone marrow toxicity occurred. Therefore, target organ exposure was demonstrated.

3)

Previous evaluation:	None – submitted for purpose of renewal (study owned by Bayer Task force)

Study ID	B.6.4.2.1/03
Study title	Micronucleus test of Orius technical in bone marrow cells of the NMRI mouse by oral administration
Test substance	Tebuconazole (orius technical)
Purity (%) **Batch no.**	98.2 Batch no: 363-036-02
Test animals	Male and female NMRI mice
Groups	5/sex/dose group
Doses	0 - 500 – 1000 – 2000 mg/kg/day
Route	Single oral dose by gavage
Vehicle	0.8 % aqueous hydroxypropylmethylcellulose
GLP	Yes
Guideline	OECD guideline 474
Deviation	The following deviations from the OECD-Guideline 474 (2016) occurred: - Only 2000 polychromatic erythrocytes scored for micronuclei - No justification for single treatment
Acceptable	Acceptable - supplementary, as a new, modern study is available.
Result	Tebuconazole tested up to the highest reasonable dose level of 2000 mg/kg b.w. by oral administration showed no mutagenic properties in the mouse bone marrow micronucleus study at the two tested sampling times of 24 hours and 48 hours.

Methods
A range finding study was first conducted, in which male and female mice (1 mouse/sex/group) were orally administered 500 – 2000 mg/kg tebuconazole. Based on the findings in this range finding study, tebuconazole was administered in two doses of 500, 1000 and 2000 mg/kg/day in a volume of 20 mL/kg in the main test. The vehicle served as the negative, and CPA as the positive control. The animals were sacrificed 24 h after the administration of the last treatment in all groups, and at 48 h after treatment in the high dose group only. For each animal, 2000 polychromatic erythrocytes were evaluated for micronuclei. The polychromatic (PE) and normochromatic (NE) erythrocyte ratio was established by scoring a total of 1000 erythrocytes (PE+NE).

Results
Clinical signs and mortality
No signs of systemic toxicity were noted in the low and intermediate dosed mice. The animals treated with 2000 mg tebuconazole/kg bw revealed slightly to moderately reduced motility, slight to moderate ataxia, slightly

reduced muscle tone and slight dyspnoea in all animals 30 minutes to 6 hours after administration.

Administration of tebuconazole did not lead to any biologically relevant increase in the number of polychromatic erythrocytes that contained micronuclei. The rate of micronuclei was close to the concurrent negative controls with no statistically significant differences observed (chi^2 test).

The formation of erythrocytes was not affected at all doses tested for samples collected 24 hours after administration. Bone marrow toxicity was noted in the high dose-treated animals with a sampling time of 48 hours after administration where the PE/NE ratio in males and females combined was decreased to 0.11. This inhibition of erythropoiesis indicated bone marrow toxicity and therefore exposure of the bone marrow to tebuconazole and its metabolites.

Table 6.4-16. Induction of nuclei in bone marrow cells (means ± SD; 24 h after last treatment)

Sampling time		Vehicle	Tebuconazole			Cyclophosphamide
	Dose (mg/kg)	0	500	1000	2000	27 mg/kg
males						
24 h	MPE/ 1000 PE	1.8	1.5	1.7	1.9	22.9
	PE/NE ratio[#1]	0.53	0.49	0.36	0.43	0.61
48 h	MPE/ 1000 PE	1.9	-	-	1.9	-
	PE/NE ratio[#1]	0.76	-	-	0.13	-
females						
24 h	MPE/ 1000 PE	1.0	1.8	1.7	1.9	19.3
	PE/NE ratio[#1]	0.51	0.50	0.32	0.38	0.53
48 h	MPE/ 1000 PE	2.0	-	-	2.8	-
	PE/NE ratio[#1]	0.77	-	-	0.09	-

MPE: Micronucleated Polychromatic Erythrocytes
PE: Polychromatic Erythrocytes
NE: Normochromatic Erythrocytes
[#1]: per 1000 counted cells

Table 6.4-17. Historical vehicle control data (mouse)

Sex		Group mean ratio PCE/NCE	Group mean frequency of micronucleated PCE (per 1000) #2	Animals (%) with 0, 1 or more micronucleated PCE (per 1000) #3						
				0	1	2	3	4	5	>6
m	Mean	0.76	1.93	7.7	29.3	30.0	19.3	8.3	4.7	0.7
	Range	0.33 - 1.25	0.2 - 3.8							
f	Mean	0.73	1.79	11.3	27.0	32.7	17.7	6.7	4.0	0.7
	Range	0.31 - 1.21	0.0 - 4.0							

#2 Average of group means from the most recent background data
Data from 24, 48 and 72 hour samplings are combined

#3 Individual animal profile based on the above experiments; data from 300 animals

m male

f female

PCE polychromatic erythrocytes

NCE normochromatic erythrocytes

In the two vehicle control groups, the frequency of MPE was consistent with the historical data. The positive control for clastogenicity, CPA, led to the expected significant increase (both sexes combine 15 times higher than vehicle control) in the rate of polychromatic erythrocytes that contained micronuclei. The sensitivity and validity of the test system under the experimental conditions was therefore confirmed.

Conclusion
Tebuconazole was not mutagenic in the somatic *in vivo* mouse micronucleus test performed in accordance with OECD guideline 474 when administered as a single oral dose from 500 to 2000 mg/kg bw to male and female NMRI mice. Bone marrow toxicity was seen at 2000 mg/kg bw in animals sacrificed at 48 hours post-dosing, providing g evidence of target organ exposure.

4)

Previous evaluation:	None – submitted for purpose of renewal (study owned by EU Tebuconazole Task Force)

Study ID	B.6.4.2.1/04
Study title	Tebuconazole technical: CD1 mouse *in vivo* micronucleus test
Test substance	Tebuconazole technical
Purity (%) **Batch no.**	Min. 97.8 Batch no: 1106613
Test animals	CD1 mice
Groups	Preliminary tests: 2 mice/sex /dose Main tests: 5 mice/sex /dose* * two additional animals per sex were used in the 1st main test high dose group (2000 mg/kg /day)
Doses	First preliminary test: 1750, 2000 mg/kg /day Second preliminary test: 750, 875 mg/kg /day First main test: 1000, 1750, 2000 mg/kg /day Second main test: 187.5, 375, 750 mg/kg /day
Route	Orally by gavage

Vehicle	methylcellulose, 1%
GLP	Yes
Guideline	OECD guideline 474
Deviation	The following deviations from the OECD-Guideline 474 (2016) occurred: - None
Acceptable	Acceptable
Result	Tebuconazole technical did not show any evidence of causing an increase in the induction of micronucleated polychromatic erythrocytes, in male and female CD1 mice when administered orally by gavage in this *in vivo* test procedure. However, bone marrow cell toxicity was observed at all dose levels tested.

Methods
A range finding study was first conducted, in which male and female mice (2 mouse/sex/group) were orally administered 1750 or 2000 mg/kg tebuconazole. Based on the findings in this study, tebuconazole was administered in two doses of either 1000, 1750 and 2000 mg/kg/day, orally by gavage approximately 24 hours apart. The vehicle served as the negative, and Mitomycin C as the positive control. The first main micronucleus test did not meet the acceptance criteria: a number of animals administered Tebuconazole technical at 1000, 1750 and 2000 mg/kg/day were killed in extremis due to the severity of the clinical signs observed leading to insufficient animal numbers in each group. Subsequently, a second micronucleus test was performed.

On the basis of the results obtained in the additional preliminary work (2 mice/sex/dose administered 750 or 875 mg/kg/day), two doses of either 187.5, 375, or 750 mg/kg/day were administered, orally by gavage approximately 24 hours apart, to both male and female animals. The animals were sacrificed 24 h after the administration of the last treatment. For each animal, 2000 polychromatic erythrocytes were evaluated for micronuclei. The proportion of polychromatic erythrocytes was assessed by examination of at least 1000 erythrocytes from each animal. A record of the incidence of micronucleated normochromatic erythrocytes was also kept.

Results
Administration of tebuconazole did not lead to any biologically relevant increase in the number of polychromatic erythrocytes that contained micronuclei. The rate of micronuclei was close to the concurrent negative controls with no statistically significant differences observed in either sex.

The proportion of polychromatic erythrocytes was statistically significantly decreased at all doses tested in males and females. As group mean treatment values for the proportion of polychromatic erythrocytes are below the current historical control data the result is considered to be biologically significant. This inhibition of erythropoiesis indicated bone marrow toxicity at all dose levels tested and therefore exposure of the bone marrow to tebuconazole and/or its metabolites.

Table 6.4-18. Induction of nuclei in bone marrow cells (means ± SD; 24 h after last treatment)

Males				
Sampling time after 2nd dose	**treatment**	**Dose (mg/kg /day)**	**Proportion of PCE (%)**	**Incidence MPCE (mean)**
24 hours	Vehicle	-	50.2	2.6
	Tebuconazole technical	187.5	31.1**++	1.2
		375	27.3**+++	1.2
		750	20.1**+++	2.0
	Mitomycin C	12	45.0**	89.2**
Females				
Sampling time after 2nd dose	**treatment**	**Dose (mg/kg /day)**	**Proportion of PCE (%)**	**Incidence MPCE (mean)**
24 hours	Vehicle	-	54.5	1.6
	Tebuconazole technical	187.5	34.5**++	0.8
		375	30.3**+++	1.4
		750	26.5**+++	1.3
	Mitomycin C	12	45.6**	80.6**

** $p < 0.01$ (WilcSipcam Oxon or Cytel pairwise test); ++ $p < 0.01$ (Jonckheere trend test); +++ $p < 0.001$ (Jonckheere trend test)

PCE polychromatic erythrocytes; MPCE number of micronucleated polychromatic erythrocytes observed per 2000 polychromatic erythrocytes examined

In the two vehicle control groups, the frequency of MPE was consistent with the historical data. The positive control for clastogenicity, Mitomycin C, led to the expected highly significant increase ($p < 0.01$) in the rate of polychromatic erythrocytes that contained micronuclei, and a statistically significant decrease in the proportion of polychromatic erythrocytes ($p<0.01$). The sensitivity and validity of the test system under the experimental conditions was therefore confirmed

Conclusion
Tebuconazole was not mutagenic in the somatic *in vivo* mouse micronucleus test performed in accordance with OECD guideline 474 when administered in two oral doses, with a 24-hour interval, from 187.5 to 750 mg/kg bw to male and female Crl:CD-1 mice. The tested doses caused bone marrow toxicity, providing evidence of target organ exposure.

B.6.4.3. *In vivo* studies in germ cells

B.6.4.3.1. ***Dominant lethal test on mice***

One dominant lethal assay is available. This was considered in the original DAR (2006).

Previous evaluation:	In Tebuconazole DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.4.3.1/01
Study title	HWG 1608 - Dominant lethal test on the male mouse to evaluate for mutagenic effect
Test substance	(HWG 1608) Tebuconazole
Purity (%) **Batch no.**	93.5 16007/83
Test animals	NMRI mice
Groups	50 males/dose group. 600 Females remained untreated
Doses	Negative control and 2000 mg/kg bw – single oral dose
Route	Oral gavage
Vehicle	1% Cremophor emulsion
GLP	Yes
Guideline	OECD guideline 478
Deviation	The following deviations from the OECD-Guideline 478 (2016) occurred: None
Acceptable	Acceptable – supplementary as non-standard test
Result	The dominant lethal test on the male NMRI mouse of a single oral dose of 2000 mg tebuconazole/kg bw did not indicate a mutagenic effect of the substance.

Methods
Tebuconazole, suspended in 1% Cremophor emulsion, was evaluated for mutagenic effects in the dominant lethal test after a single oral treatment (by gavage) of male NMRI mice with 0 and 2000 mg/kg bw. Dose levels were selected on basis on a pilot test with dose levels from 500 - 3000 mg/kg in male mice with 5 mice/group: Two of the animals treated with 3000 mg/kg died, and all were slightly drowsy and had bristling coats. 600 untreated female mice per group were mated with the 50 males. The administered volume was 10 mL/kg bw.

Results
Clinical signs and mortality
The dose tested was well tolerated. It did not lead to toxic signs or mortalities.

Fertilisation rates
The fertility of the mice was not affected by the dose used and no adverse effect on the treated male (bucks)' fertilization rates was observed.

Table 6.4-19. Fertilisation rates (mating period 1-12)

	Control group	Treated group
Females evaluated (total n)	599	600
Females fertilized (total n)	470	467
Fertilization quotes (%)	78.5	77.8

No treatment-related or statistically significant differences were reported between the control and the treatment group with respect to the parameters relevant to assessment of a mutagenic effect (corpora lutea, dead implants, live implants, total implants, pre- and post-implantation losses).

Pre-implantation loss
The pre-implantation losses, based on the distribution and variance in implantation rates and corpora lutea per female, were not affected by the test substance. Tebuconazole does not result in an increase of pre-implantation losses.

Table 6.4-20. Pre-Implantation loss (mating period 1-12)

	Control group	Treated group
Corpora lutea (total n)	6423	6436
Corpora lutea (mean n per fertilized female)	13.7	13.8
Implantations (total n)	6040	5979
Implantations (mean n per fertilized female)	12.9	12.8

Post-implantation loss
The Post-implantation losses based on the rates of live and dead implants per female, do not reveal that the substance produced an effect. Tebuconazole does not affect the post-implantation losses and the live implantation rate.

Table 6.4-21. Post-Implantation loss (mating period 1-12)

	Control group	Treated group
Living implants (total n)	5669	5581
Living implants (mean n per fertilized female)	12.1	12.0
Dead implants (total n)	375	407
Dead implants (mean n per fertilized female)	0.80	0.87

Conclusion
The dominant lethal test on the male NMRI mouse of a single oral dose of 2000 mg tebuconazole/kg bw did not indicate a mutagenic effect of the substance.

B.6.4.4. Summary of genotoxicity

The genotoxic potential of tebuconazole has been investigated in a series of *in vitro* and *in vivo* studies. A summary of the available genotoxicity studies is presented in the table below. With the exception of new studies (dated 2008 onwards), these were all evaluated in the original DAR (2006).

The following key conclusions were obtained from the evaluation of the genotoxic information:
- Tebuconazole is not genotoxic
- Classification for genotoxicity is not required
- The data requirements of Regulation 283/2013 have been met.

Test system	Concentration/ dose levels	Purity (%)	Results	Reference
***In vitro* studies**				
Pol test *E. coli* (K12)p 3478; W 3110 (+/- S-9 mix) (Bayer Task Force)	625-10 000 µg/plate	97.1	negative	B.6.4.1.1/01

Test system	Concentration/ dose levels	Purity (%)	Results	Reference
Salmonella/microsome test (TA1535, TA100, TA1537, TA98; +/– S-9 mix) (Bayer Task Force)	20-12 500 µg/plate	97.0	negative	B.6.4.1.2/03
Reverse mutation assay *S. typhimurium* (TA 98, TA 100, TA 1535, TA1537) *E.coli* (WP2/uvrA) +/– S-9 (EU Tebuconazole Task Force)	1.00-5000 µg/plate	98.8	negative	B.6.4.1.2/02
Salmonella/microsome test (TA 98, TA 100, TA 1535, TA 1537, TA1538; +/– S-9 mix) (Bayer Task Force)	37.5 - 2400 µg/plate 39.5 - 450 µg/plate	96.6	negative	Herbold (1988a)
Reverse mutation assay *S. typhimurium* (TA 98, TA 100, TA 1535, TA 1537) *E.coli* (WP2/uvrA) +/– S-9 (Bayer Task Force)	15.625-500 µg/plate (+/- S-9 mix) 31.25-1000 µg/plate (- S-9 mix) 15.625-5000 µg/plate (+ S-9 mix)	98.0	negative	B.6.4.1.2/04
Reverse mutation assay *S. typhimurium* (TA 98, TA 100, TA 102, TA 1535, TA 1537) +/– S-9 (Bayer Task Force)	plate incorporation test: 3-5000 µg/plate (+/- S-9 mix) pre-incubation test: 3-5000 µg/plate (+/- S-9 mix)	95.7	negative	B.6.4.1.2/05
Reverse mutation assay S. typhimurium (TA 98, TA 100, TA 1535, TA 1537) *E.coli* (WP2/uvrA) +/– S-9 (EU Tebuconazole Task Force)	1.5 - 5000 µg/plate	98.4	negative	B.6.4.1.2/06
Reverse mutation assay S. typhimurium (TA 98, TA 100, TA 1535, TA 1537) *E.coli* (WP2/uvrA) +/– S-9 (EU Tebuconazole Task Force)	1.5 - 5000 µg/plate	97.21	negative	B.6.4.1.2/07
Cytogenetic *in vitro* (human lymphocytes) (Bayer Task Force)	3-30 µg/mL (- S-9 mix) 30-300 µg/mL (+ S-9 mix)	96.5 – 96.6	negative	B.6.4.1.3/01
CHO/HGPRT (Bayer Task Force)	80 - 100 µg/mL (- S-9 mix) 12.5 - 200 µg/mL (+ S-9 mix)	96.6	negative	B.6.4.1.5/01
CHO/HGPRT (Bayer Task Force)	3.9 – 250 µg/mL (+/- S-9 mix)	95.7	negative	B.6.4.1.5/02

Test system	Concentration/ dose levels	Purity (%)	Results	Reference
SCE/CHO (Bayer Task Force)	4 - 30 µg/mL (- S-9 mix) 15 - 120 µg/mL (+ S-9 mix)	96.5	negative	B.6.4.1.6/01
Primary rat hepatocyte UDS (Bayer Task Force)	0.504 - 25.2 µg/mL	96.5	negative	B.6.4.1.7/01
Rec-assay (*B. subtilis* H17, M45) (Bayer Task Force)	0.313 - 20 µg/plate (+/- S-9 mix)	98.0	negative	B.6.4.1.8/01
Micronucleus test *in vitro*, (human lymphocytes) (Bayer Task Force)	21.9-122 µg/mL (- S9 mix) 39.8-122 µg/mL (+ S9 mix)	95.7	negative	B.6.4.1.4/01
***In vivo* studies**				
Micronucleus test (male and female NMRI mice) Oral, gavage (Bayer Task Force)	200-2000 mg/kg bw	95.3	negative (PE/NE ratio altered)	B.6.4.2.1/01
Micronucleus test (male and female Crl:CD-1 (ICR) BR mice) Oral (Bayer Task Force)	187.5-750 mg/kg/day (total doses: 375-1500 mg/kg)	99.5	negative (PE/NE ratio altered)	B.6.4.2.1/02
Micronucleus test (male and female NMRI mice) Oral, gavage (Bayer Task Force)	500-2000 mg/kg	98.2	negative (PE/NE ratio altered)	B.6.4.2.1/03
Micronucleus test (male and female CD1 mice) Oral, gavage (EU Tebuconazole Task Force)	1000- 2000 mg/kg /day 187.5-750 mg/kg /day	97.8	negative (PE/NE ratio altered)	B.6.4.2.1/04
Dominant lethal test (male NMRI mice) Oral, gavage (Bayer Task Force)	2000 mg/kg bw	93.5	negative	B.6.4.3.1/01

Tebuconazole was negative in an extensive number of *in vitro* and *in vivo* studies to investigate its genotoxic potential.

Tebuconazole was negative when tested up to cytotoxic concentrations in numerous Ames tests and in multiple HPRT-locus mammalian cell mutation assays in CHO and V79 cells. A supplementary *in vitro* rat liver UDS assay was also negative. Tebuconazole did not induce chromosome aberrations in human lymphocytes, or significant increases in the frequency of sister chromatid exchanges in CHO cells, in the presence and absence of metabolic activation up to cytotoxic concentrations. Also, it was clearly not clastogenic or aneugenic to human lymphocytes in a new, guideline compliant *in vitro* micronucleus assay conducted up to cytotoxic concentrations. Overall, there was no evidence of genotoxicity across these *in vitro* studies.

Four *in vivo* mouse bone-marrow micronucleus assays, all via oral administration, were available. In all of these, no increase in the incidence of micronuclei was induced. The assays were compliant with the contemporary OECD guideline 474. Bone-marrow toxicity was demonstrated in these assays (reduced PCE/NCE ratio). A dominant lethal test in mice with a single oral dose of 2000 mg /kg bw did not indicate a mutagenic effect of the substance. Overall, there was no evidence of genotoxicity across these *in vivo* studies.

According to Regulation (EU) 283/2013, photo-mutagenicity testing is not required for substances with a UV/VIS molar extinction/absorption coefficient less than 1000 L x mol^{-1} x cm^{-1}. There is no relevant absorption in the range 290 - 700 nm and the ultraviolet/visible molar extinction/absorption coefficient of the active substance is less than 10 L x mol^{-1} x cm^{-1} (see chemistry evaluation section B.2.4). Photo-mutagenicity testing is therefore not required for tebuconazole.

Overall, the RMS concludes that tebuconazole was not genotoxic *in vitro* or *in vivo* in a series of investigations that, together, meet the data requirements of Regulation 283/2013. Consideration of the results of the original and newly-submitted studies against the CLP criteria has confirmed the previous conclusion (classification is not required for germ cell mutagenicity).

B.6.5. LONG-TERM TOXICITY AND CARCINOGENESIS

A total of three guideline oral combined chronic toxicity and carcinogenicity studies were described in the original DAR (2006), one in the rat (B.6.5.1/01) and two in the mouse (B.6.5.2/01; and B.6.5.2/02). All were conducted according to GLP and OECD test guidelines (available at the time the study was conducted) and were considered to be acceptable at the time. An evaluation of deviations, as well as relevant impact of deviations, to current OECD test guidelines has been conducted for each study.

B.6.5.1. Studies in rats – combined chronic and carcinogenicity

Previous evaluation	In DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.5.1/01
Study title	HWG 1608 – Study for chronic toxicity and cancerogenicity in Wistar rats (Administration in diet for two years) Addendum: The incidence of Thyroid Follicular Adenomas, C-Cell Adenomas and Carcinomas, and C-Cell Hyperplasias – A Compilation of Historical data
Dates (in life)	29 October 1984 – 31 October 1986
Test substance	Tebuconazole
Purity (%) **Batch no.**	> 95 Mixed batch Fl. no.: 132 Single samples: 16001/84, PAV 994, 97.7%;16002/84, PAV 995, 95.6%; 16003/84, PAV 996, 95.8%; 16004/84, PAV 997, 96.2%; 16006/84, PAV 998, 98.3%
Test animals	Wistar Bor:WISW (SPF-Cpb) rats
Groups	60/sex/group
Dose	0, 100, 300 or 1000 ppm (equivalent to 0, 5.3, 15.9 or 55.0 mg/kg bw/day for males and 0, 7.4, 22.8 or 86.3 mg/kg bw/day for females)
Route	Oral, dietary
Vehicle	Plain diet; no positive control.
GLP	Yes
Guideline	OECD-Guideline 453 (1981) Note that the current guideline was adopted in 2009
Deviation	The following deviations from the current OECD-Guideline 453 (2009) occurred: - Ophthalmological examination covered only 10 males and 10 females in control and high dose group instead of all animals of those groups - Haematological examination and urinalysis at 3 months after study initiation is missing, (According to the guideline *"Measurements at 3 months […] need not be conducted if no effect was seen on haematological parameters in a previous 90 day study carried out at comparable dose levels."* This data point can be covered by B.6.3.2.1/01- a 90-day study conducted with 0, 100, 400 and 1600 ppm in the same rat strain (B.6.3.2). - Weight of the following organs was not determined: epididymides, thyroid (incl. parathyroids) and uterus

	- The following tissues were not subject to histopathological examination: cervix, lacrimal gland, bone marrow
Impact of deviations	Minor – the deviations are minimal, can be compensated by the results of other studies and thus they are not considered to affect the validity of the study.
Acceptable	Acceptable
NOAEL	Carcinogenicity: 1000 ppm for males and females (equivalent to 55 mg/kg bw/day for males and 86.3 mg/kg bw/day for females respectively) (top dose) Systemic toxicity: 300 ppm for females (equivalent to 23 mg/kg bw/day) and 1000 ppm (top dose) for males (equivalent to 55 mg/kg bw/day)
Effects at the LOAEL	Carcinogenicity: No carcinogenic effects observed. Systemic toxicity: Lower body weight gain in females and histopathological findings of the adrenal, spleen and liver in females at the top dose of 1000 ppm. No significant systemic toxicity in males up to the top dose.

Methods
Groups of 50 male and 50 female rats were given tebuconazole at concentrations of 0, 100, 300 and 1000 ppm (doses in mg/kg bw/day are provided in Table 6.5-1.) for two years in their diet. Ten similarly treated male and female animals (satellite groups) were sacrificed after a study period of twelve months. The tebuconazole doses were based on the results of a previous feeding study with Wistar rats of the same strain lasting thirteen weeks (B.6.3.2.1/01) (see section B.6.1.1.3.). Tebuconazole was administered to the animals in the treatment groups from start of study until spontaneous death or time of sacrifice, for *ad-libitum* consumption in the diet. The animals were inspected at least twice daily, and any clinical signs and special features were noted. Detailed individual inspections took place once a week. Body surfaces, orifices, posture, general behaviour, respiration and excretory products were assessed. Ophthalmological examinations were made at the start of the study, after 12 months and before end of study, covering groups of ten males and ten females in the control group and the 1000 ppm dose group. Individual body weight was recorded weekly for the first 13 weeks and once every two weeks thereafter. Food and water intake were determined group wise from start of the study up to and including week 13 once a week, and from week 15 every two weeks. Laboratory examinations of blood and urine were made after 6, 12, 18 and 24 months of ten animals per group.

Animals which died spontaneously during the study or were moribund and sacrificed were dissected and their organs/tissue subjected to detailed gross pathological examination. After 12 and 24 months all the survivors of the satellite groups and main groups respectively were sacrificed and autopsied. The organs/tissue of the dissected animals was subjected to detailed gross pathological examination.

Table 6.5-1. Study design and dose received

Test group		**1**	**2**	**3**	**4**
Concentration in diet	(ppm)	**0**	**100**	**300**	**1000**
Dose per animal [mg/kg bw/day]	Male	0	5.3	15.9	55.0
	Female	0	7.4	22.8	86.3

Results
Clinical observations
The appearance, and general behaviour were unaffected by treatment. Mortality was not affected by treatment.

Ophthalmoscopic results
The ophthalmological and histopathological examinations did not provide any indications of substance-induced damage to the eye.

Body weight and food intake
The growth development of females in the 1000 ppm (top) dose group was reduced. Changes in body weight gain of males at 1000 ppm and females at 300 ppm were not significantly different from controls (< 10 % change vs. control) (Table 6.5-2). 1000 ppm females had reduced water intake (-5 %) and increased food consumption (+15 %); however, these effects were not statistically significant and were not considered adverse. Overall, treatment-related effects on body weights were seen in females at the top dose.

Table 6.5-2. Intergroup comparison of body weights / gain (g) of main groups – selected time points

Dose [ppm]	wk	Males				Females			
		0	**100**	**300**	**1000**	**0**	**100**	**300**	**1000**
Body weight [g]	0	99	99	**96***	**95****	91	90	89	90
	27	374	383	371	369	222	220	**212****	**202****
	53	399	409	404	390	239	238	229	**219****
	79	412	426	419	411	260	254	**243****	**234** (-10%)**
	104	396	404	413	387	253	264	259	248
Body weight gain [g] *(% change vs control)*	0-27	275	284 *(+ 3)*	275 *(± 0)*	274 *(± 0)*	131	130 *(- 1)*	123 *(- 6)*	112 *(- 14.5)*
	0-53	300	310 *(+ 3)*	308 *(+ 3)*	295 *(- 2)*	148	148 *(± 0)*	140 *(- 5)*	129 *(- 13)*
	0-104	297	305 *(+ 3)*	317 *(+ 7)*	292 *(- 2)*	162	174 *(+ 7)*	170 *(+ 5)*	158 *(- 2)*
Mean food consumption [g/kg bw/day]		54.6	52.8	53.1	55.0	74.8	73.7	76.1	86.3 *(+ 15)*
Mean Water consumption [g/kg bw/day]		72.4	71.4	70.2	71.2	105.0	105.4	103.2	99.6 *(- 5)*

* statistically significant difference from control $p \leq 0.05$
** statistically significant difference from control $p \leq 0.01$
Differences to control in % in brackets only if major differences observed
Statistically significant values are written in bold letters.

Haematology and clinical chemistry

Haematological findings

The haematological examinations did not provide any indication of damage to the blood (Table 6.5-3). As any statistically significant findings were not consistent across time points and did not reveal a dose-relationship they were not considered treatment-related. Overall, there were no adverse, treatment-related effects on haematological parameters up to the top dose.

Table 6.5-3. Haematology results

Haematology											
Dose [ppm]	**Week**	**LEUCO [10^9/L]**	**ERY [10^{12}/L]**	**HB [g/L]**	**HCT [L/L]**	**MCV [fL]**	**MCH [pg]**	**MCHC [g/L ERY]**	**RETI [0/00]**	**THRO [10^9/L]**	**HQUICK [sec]**
Males											
0	27	7.1	8.24	157	0.442	54	19.2	351	20	1090	32.6
100	27	6.5	8.39	156	0.447	54	18.7	345	24	1022	31.3
300	27	6.5	8.51	158	0.445	53	18.7	351	21	1135	32.3
1000	27	6.8	**8.76** (+6.3 %)**	160	0.458	53	**18.4** (-4.2 %)**	**345***	**26** (30 %)**	1100	32.3
Females											
0	27	5.9	7.93	158	0.457	58	20.0	342	17	947	28.8
100	27	5.7	7.83	156	0.457	59	20.1	338	17	999	29.6
300	27	5.7	7.99	156	0.454	57	19.7	340	18	1020	29.1
1000	27	5.3	8.09	155	0.450	**56***	**19.4***	342	14	984	28.6
Males											
0	52	6.9	8.80	151	0.474	55	18.2	308	16	1041	35.3

Haematology											
Dose [ppm]	**Week**	**LEUCO [10^9/L]**	**ERY [10^{12}/L]**	**HB [g/L]**	**HCT [L/L]**	**MCV [fL]**	**MCH [pg]**	**MCHC [g/L ERY]**	**RETI [0/00]**	**THRO [10^9/L]**	**HQUICK [sec]**
100	52	6.3	8.59	**147***	**0.459***	55	18.1	309	16	1040	33.1
300	52	7.1	8.93	151	0.470	54	17.9	310	17	1113	32.4
1000	52	7.4	8.90	149	0.475	55	17.6	302	15	1113	33.4
Females											
0	52	5.1	7.43	141	0.438	61	20.2	310	17	1141	29.6
100	52	5.3	7.43	143	0.427	59	20.3	324*	16	1187	28.9
300	52	5.5	7.50	141	0.423	59	20.0	321*	16	1170	28.8
1000	52	5.3	7.52	146	0.418*	57**	20.5	336**	14*	1135	29.6
Males											
0	79	7.2	8.46	157	0.496	59	18.5	319	23	864	31.2
100	79	12.5	8.22	153	0.482	59	18.5	319	17*	829	30.2
300	79	7.7	8.40	153	0.482	57	18.2	320	21	942	28.0
1000	79	8.1	8.47	153	0.482	57	18.0	319	19	861	32.4
Females											
0	79	6.2	7.25	149	0.466	65	20.5	322	23	754	31.0
100	79	5.6	7.35	148	0.460	63	19.9	324	25	809	28.0**
300	79	6.3	7.39	145**	0.452**	61**	19.5*	323	22	772	28.1**
1000	79	6.1	7.50	144*	0.453*	60**	19.1**	321	21	738	28.9
Males											
0	104	7.5	7.87	147	0.459	58	18.6	319	22	893	33.4
100	104	7.0	8.13	152	0.472	58	18.6	325	20	957	31.1*
300	104	6.9	7.93	146	0.460	58	18.3	315	20	956	32.4
1000	104	7.9	8.43	151	0.478	57	17.9	315	21	891	34.2
Females											
0	104	7.3	7.20	146	0.454	63	20.3	321	23	894	31.4
100	104	5.7	7.55	148	0.453	60	19.4	324	24	889	31.4
300	104	5.1	7.44	143	0.441	59	19.1*	321	21	801	31.2
1000	104	5.2	7.47	143	0.446	59	19.1**	322	21	773	31.2

* statistically significant difference from control $p \leq 0.05$

** statistically significant difference from control $p \leq 0.01$

Clinical chemistry findings

The result of the clinical chemical analyses revealed changes in some parameters at all dose levels; however, due to inconsistencies between time points, direction of the change at different time points and lack of dose response, these effects were not considered treatment-related (Table 6.5-4).

Table 6.5-4. Clinical chemistry data

		Males				Females			
Dose [ppm]	**wk**	**0**	**100**	**300**	**1000**	**0**	**100**	**300**	**1000**
ASAT [U/L]	27	45.1	41.2	39.1* ↓	43.6	42.9	39.7	40.3	39.5

Dose [ppm]	wk	Males 0	Males 100	Males 300	Males 1000	Females 0	Females 100	Females 300	Females 1000
	52	35.2	37.4	39.6* ↑	**64.3**** ↑	36.9	46.9	42.1	41.2
	79	37.4	40.0	37.5	40.5	54.5	77.0	67.6	68.7* ↑#
	104	39.6	33.8	38.8	42.4	36.9	46.0	37.0	38.8
ALAT [U/L]	27	28.8	26.4	27.4	30.5	26.4	22.5	22.3	24.4
	52	27.9	29.1	30.6	**38.2*** ↑	26.9	29.5	30.3	28.2
	79	49.8	45.2	51.6	52.9	50.8	52.8	56.0	**65.0**** ↑
	104	46.5	47.9	52.9	**58.6*** ↑	42.2	49.8* ↑	41.5	47.3
APh [U/L]	27	122	127	120	126	67	62	71	71
	52	121	124	130	115	63	56	61	64
	79	177	176	168	169	112	93	108	119
	104	151	149	157	160	115	114	99	105
LDH [U/L]	27	86	82	84	69	169	127* ↓	96** ↓	65** ↓
	52	88	101	117	1093** ↑#	126	250	157	105
	79	184	174	162	169	447	1281** ↑#	804	706** ↑#
	104	176	168	117** ↓	124	108	115	95	102
CK [U/L]	27	68	89	92	87	91	58* ↓	39** ↓	35** ↓
	52	49	47	59	226 ↑#	45	70	52	38
	79	66	58	46	52	124	282* ↑#	262* ↑#	286** ↑#
	104	148	81	68	70	63	52	63	**110*** ↑
Triglycerides [mmol/L]	27	0.59	0.63	0.76	0.57	0.40	0.37	0.35	**0.31*** ↓
	52	0.95	0.78	1.04	0.70* ↓	0.59	0.64	0.45* ↓	**0.43**** ↓
	79	1.88	2.00	2.04	2.02	1.33	1.71	1.32	1.16
	104	2.49	3.17	2.09	1.88	1.45	2.03	1.42	1.07

wk: week
* statistically significant difference from control p≤0.05
** statistically significant difference from control p≤0.01
# These high activities may not be interpreted as a toxicologically relevant treatment-induced effect, because Activity of these enzymes has shown to depend greatly on the blood sampling technique used and in addition a dose correlation is absent
ASAT: Aspartate aminotransferase ALAT: Alanine aminotransferase APh: Alkaline phosphatase
LDH: Lactate dehydrogenase CK: Creatine kinase

Urinalyses
The result of urinalyses did not reveal any toxicologically-relevant effects. As any statistically significant findings occurred in isolation, were not consistent across time points and did not reveal a dose-relationship they were not considered treatment-related. Overall, there were no adverse, treatment-related effects on urinalysis parameters.

Table 6.5-5. Urinalysis data

Dose [ppm]	Week	Urinalysis VOL [mL]	DENSITY [g/L]	PROT [g/L]	PROT+VOL [mg]
Males					
0	27	5	1042	1.58	5.8
100	27	6	**1026***	1.51	8.6
300	27	5	1029	1.59	8.4
1000	27	4	1034	1.49	5.3
Females					
0	27	6	1019	0.39	2.4
100	27	6	1018	0.33	2.1
300	27	5	1019	0.31	**1.5****
1000	27	**5***	1021	0.30	**1.5****

Urinalysis					
Dose [ppm]	**Week**	**VOL [mL]**	**DENSITY [g/L]**	**PROT [g/L]**	**PROT+VOL [mg]**
Males					
0	52	5	1045	1.72	7.8
100	52	6	1042	2.46	**12.2***
300	52	5	1047	1.71	8.9
1000	52	5	1038	1.86	8.5
Females					
0	52	6	1031	0.33	1.6
100	52	7	1023	0.34	1.7
300	52	7	1022	0.21	1.2
1000	52	7	1020	**0.17***	**1.0***
Males					
0	79	3	1074	4.32	11.6
100	79	**4****	**1043****	4.73	18.0
300	79	3	**1045***	2.82	7.9
1000	79	3	1059	3.14	8.3
Females					
0	79	8	1022	0.62	3.9
100	79	9	1020	0.76	5.3
300	79	7	1021	0.37	2.1
1000	79	11	1013	**0.21****	2.2
Males					
0	104	6	1034	2.28	16.1
100	104	6	1036	**4.46***	**25.8***
300	104	6	1038	2.97	17.7
1000	104	6	1037	2.64	16.5
Females					
0	104	5	1031	0.93	4.4
100	104	7	1022	1.23	8.2
300	104	5	1028	1.28	5.8
1000	104	7	1023	**0.31****	**2.0***

* statistically significant difference from control p≤0.05
** statistically significant difference from control p≤0.01
Statistically significant values are written in **bold letters**.

Organ weights
The females' absolute and relative spleen weights in the 1000 ppm group were statistically significantly increased after 12 months (Table 6.5-6). On final autopsy after 24 months a similar effect was no longer present. However, given the associated histopathology (see below), these increased spleen weights in the 1000 ppm females were considered treatment-related and adverse.

At the final autopsy the female animals' absolute and relative adrenal weights in all the treatment groups were slightly lower than the controls' weights. A clear dose-response relationship was not observed; however, at the top dose, histopathological findings were noted (see below). On this basis, the reduced adrenal weights in females at the top dose were considered treatment-related and adverse.

After 12 months females' absolute and relative liver weight in the 1000 dose group were reduced, at a statistically significant level. However, this was not evident after 24 month in females in the 1000 ppm dose group. Therefore this observation is not considered to be treatment-related or adverse.

After 24 months the males' relative testicle weights in the main 1000 ppm treatment groups were statistically significantly lower than the control males'. The mean value in the control group was however unusually high, due to individual extreme figures caused by tumours. Therefore, these changes in testicle weights were not considered related to treatment.

The other organ weight differences were relatively slight, partly without dose correlation, and/or could be explained by variations in the body weights. Overall, treatment-related and adverse changes in the weight of the spleen and adrenal were seen in the top dose females.

Table 6.5-6. Organ, weights absolute and relative, (± % change compared with control)

Organ weights, absolute [mg]										
Dose [ppm]	**Week**	**Body weight [g]**	**Brain**	**Heart**	**Lungs**	**Liver**	**Spleen**	**Kidneys**	**Adrenals**	**Testes /Ovaries**
Males										
0	53	418	1987	1380	1369	14437	636	2596	39	3896
100	53	423	1989	1299	1382	13856	672	2477	41	3726
300	53	398	1940	**1220***	1334	13126	622	2409	36	3551
1000	53	**384***	1938	1286	1368	12881	636	2485	37	3576
Females										
0	53	229	1813	858	965	8638	430	1587	62	122
100	53	227	1749	910	971	8140	462	1587	62	123
300	53	233	1807	857	970	8048	416	1571	59	114
1000	53	232	1853	907	**1116***	**7812* (-9.6 %)**	**504** (+17.2 %)**	1568	60	120
Males										
0	104	400	2064	1429	1517	14760	814	2791	51	3880
100	104	403	2062	1512	1596	14549	832	2737	53	3807
300	104	407	2020	1470	1563	14448	802	2741	46	3619
1000	104	395	2025	1444	1469	14256	783	2693	47	3489
Females										
0	104	259	1881	1144	1156	9176	548	1869	78	142
100	104	260	1886	1137	1194	9248	561	1914	**65* (-16.7 %)**	142
300	104	252	1881	1129	1134	8843	549	1887	**64** (-17.9 %)**	138
1000	104	**242** (-6.6 %)**	1878	1076	1148	9108	562	1817	**57** (-26.9 %)**	137
Organ weights, relative [mg/100 g]										
Males										
0	53	418	478	331	328	3447	153	621	9	932
100	53	423	471	307	328	3269	159	587	10	884
300	53	398	491	307	337	3305	156	605	9	896
1000	53	**384***	505	335	356	3361	166	647	9	932
Females										
0	53	229	793	375	422	3769	188	696	27	53

100	53	227	771	402	428	3585	**203***	699	27	54
300	53	233	778	368	416	**3454* (-8.4 %)**	178	674	25	49
1000	53	232	801	390	**482* (+14.2 %)**	**3365** (-10.7 %)**	**217** (+15.4 %)**	677	26	52
Males										
0	104	400	520	360	381	3700	204	703	13	980
100	104	403	515	379	399	3613	207	681	13	952
300	104	407	502	365	387	3555	199	677	11	887
1000	104	395	517	368	375	3620	200	685	12	**883***
Females										
0	104	259	735	446	451	3567	215	729	31	55
100	104	260	733	440	464	3550	216	741	**25* (-19.4 %)**	57
300	104	252	753	452	454	3504	220	751	**26* (-16.1 %)**	55
1000	104	**242****	**783****	447	478	**3773***	233	753	**24** (-22.6 %)**	57

* statistically significant difference from control p≤0.05
** statistically significant difference from control p≤0.01
Statistically significant values are written in **bold letters**.

Histopathology
Non-neoplastic findings
The gross pathological and histopathological examinations did not provide any indications of substance-induced organ lesions (Tables 6.5-7. & 6.5-8.).

The histopathological examination did not detect any effects on the adrenals in the male animals in all dose groups or in females in dose groups up to and including 300 ppm. However, at 1000 ppm, there was a clearly reduced number of females with haemorrhagic degeneration of the adrenal cortex.

Histopathology revealed an increased incidence of females with haemosiderin accumulation in the spleen and pigment deposits in the Kupffer star cells in the liver in the 1000 ppm dose group.

Table 6.5-7. Group Incidences of Histopathology Findings 53 week interim sacrifice

Organ/lesion	**Males**				**Female**			
	Control	**100 ppm**	**300 ppm**	**1000 ppm**	**Control**	**100 ppm**	**300 ppm**	**1000 ppm**
Liver								
Samples examined	9	10	10	10	10	10	10	10
Clear cell focus (1)	5	5	3	7	1	-	1	-
Bile duct hyperplasia (sclerotic)	-	1	-	-	-	-	-	-
Microfoci Inflammatory cells	-	-	-	1	-	-	-	-
Subscapular cyst	-	-	-	-	1	-	-	-
Adrenals								

Samples examined	10	10	10	10	10	10	10	10
Angiectasis (cortex)	-	-	-	-	4	3	3	-
Degeneration (cortex)	-	-	-	-	1	-	2	-

Table 6.5-8. Group Incidences of Histopathology Findings 104 week sacrifice - Males

Organ/Lesion	**Males**											
	Control			**100 ppm**			**300 ppm**			**1000 ppm**		
	T	**TK**	**PD**	**T**	**TK**	**PD**	**T**	**TK**	**PD**	**T**	**TK**	**PD**
Liver, samples evaluated	49	41	8	49	41	8	50	42	8	50	47	3
Angiectasis	0	0	0	1	1	0	0	0	0	0	0	0
Bile duct hyperplasia	0	0	0	0	0	0	0	0	0	0	0	0
Bile duct hyperplasia (sclerotic)	13	12	1	13	12	1	14	14	0	7	7	0
Cyst(s) (biliary)	1	1	0	1	1	0	2	2	0	0	0	0
Cyst(s) (subcapsular)	0	0	0	0	0	0	0	0	0	0	0	0
Congestion	4	0	4	3	0	3	2	0	2	1	0	1
Congestion (centrilobular)	1	0	1	2	0	2	1	1	0	0	0	0
Fibrosis (Centrilobular)	0	0	0	0	0	0	0	0	0	0	0	0
Focus (i) cellular change-Basophilic	4	4	0	4	3	1	3	3	0	5	5	0
Focus (i) cellular change-Clear cell	35	34	1	28	28	0	30	29	1	34	34	0
Focus (i) cellular change-Eosinophilic	0	0	0	0	0	0	0	0	0	0	0	0
Focus (i) cellular change-Mixed	0	0	0	0	0	0	0	0	0	0	0	0
Focus (i) cellular change-Pale cell	4	3	1	4	3	1	2	2	0	8	8	0
Hepatocyte degenerative change	0	0	0	0	0	0	0	0	0	0	0	0
Hemopoiesis	0	0	0	0	0	0	0	0	0	0	0	0
Kupffer cell pigmentation	0	0	0	1	0	1	0	0	0	1	0	1
Leucocytosis	0	0	0	0	0	0	0	0	0	0	0	0
Microabscess(es)	0	0	0	0	0	0	1	1	0	1	0	1
Mineralization, focal	0	0	0	0	0	0	0	0	0	0	0	0
Necrosis, foci/areas	0	0	0	1	1	0	1	0	1	1	1	0
Necrosis, centrilobular	0	0	0	0	0	0	1	0	1	0	0	0
Necrosis, single-cell	0	0	0	3	1	2	5	5	0	2	2	0
Necrosis, periportal	0	0	0	0	0	0	0	0	0	0	0	0
Peliosis hepatis focal	0	0	0	0	0	0	0	0	0	0	0	0
Spongiosis hepatis	2	1	1	1	0	1	0	0	0	0	0	0
Thrombosis	0	0	0	0	0	0	0	0	0	0	0	0
Vacuolation foci/area(s)	0	0	0	0	0	0	1	1	0	1	1	0
Vacuolation centrilobular	0	0	0	0	0	0	1	0	1	0	0	0

Organ/Lesion	Males											
	Control			100 ppm			300 ppm			1000 ppm		
	T	TK	PD	T	TK	PD	T	TK	PD	T	TK	PD
Vacuolation single-cell	0	0	0	2	2	0	1	0	1	0	0	0
Vacuolation midzonal	0	0	0	0	0	0	1	0	1	0	0	0
Peritonitis	0	0	0	0	0	0	0	0	0	0	0	0
Adrenals, samples evaluated	49	41	8	49	41	8	50	42	8	49	46	3
Medullary hyperplasia	6	5	1	5	5	0	5	5	0	7	6	1
Invaded by malignant lymphoma	0	0	0	1	0	1	0	0	0	1	1	0
Angiectasis, cortex	0	0	0	0	0	0	0	0	0	0	0	0
Haemorrhagic degeneration, cortex	3	3	0	4	3	1	4	4	0	1	0	1
Congestion	0	0	0	1	0	1	2	1	1	1	0	1
Diffuse vacuolation	0	0	0	1	1	0	1	0	1	1	1	0
Focal vacuolation, cortex	9	9	0	9	9	0	10	9	1	13	12	1
Focal eosinophilic cellular change, cortex	3	3	0	1	1	0	3	2	1	2	2	0
Haemorrhage	0	0	0	0	0	0	1	1	0	0	0	0
Extramedullary hemopoiesis	0	0	0	0	0	0	0	0	0	0	0	0
Spleen, samples evaluated	49	41	8	49	41	8	50	42	8	50	47	3
Increased hemopoiesis	2	0	2	1	0	1	1	0	1	1	0	1
Increased hemosiderin	0	0	0	0	0	0	1	0	1	0	0	0
Myelofibrosis	0	0	0	0	0	0	0	0	0	1	1	0
Lymphoid depletion	0	0	0	0	0	0	1	0	1	0	0	0
Necrosis	0	0	0	0	0	0	1	0	1	0	0	0
Peritonitis	0	0	0	0	0	0	1	1	0	0	0	0

T =Total; TK = Terminal Kill, i.e. sacrificed at end of study; PD = Intercurrent death, i.e. found dead or killed in moribund state

Table 6.5-9. Group Incidences of Histopathology Findings 104 week sacrifice - Females

Organ/Lesion	Females											
	Control			100 ppm			300 ppm			1000 ppm		
	T	TK	PD	T	TK	PD	T	TK	PD	T	TK	PD
Liver, samples evaluated	49	39	10	50	38	12	50	39	11	50	41	9
Angiectasis	0	0	0	0	0	0	0	0	0	0	0	0
Bile duct hyperplasia	0	0	0	1	0	1	2	0	2	1	0	1
Bile duct hyperplasia (sclerotic)	1	1	0	0	0	0	1	1	0	1	1	0
Cyst(s) (biliary)	1	1	0	1	1	0	1	1	0	2	1	1
Cyst(s) (subcapsular)	0	0	0	0	0	0	0	0	1	1	1	0
Congestion	4	1	3	0	0	0	1	0	1	1	0	1
Congestion (centrilobular)	1	0	1	0	0	0	0	0	0	1	0	1
Fibrosis (Centrilobular)	0	0	0	0	0	0	1	0	1			
Focus (i) cellular	15	15	0	12	12	0	12	11	0	19	17	1

Organ/Lesion	Females											
	Control			100 ppm			300 ppm			1000 ppm		
	T	TK	PD	T	TK	PD	T	TK	PD	T	TK	PD
change-Basophilic												
Focus (i) cellular change-Clear cell	8	7	1	8	8	0	7	6	1	11	10	1
Focus (i) cellular change-Eosinophilic	0	0	0	1	1	0	0	0	0	0	0	0
Focus (i) cellular change-Mixed	0	0	0	0	0	0	0	0	0	1	1	0
Focus (i) cellular change-Pale cell	0	0	0	0	0	0	1	1	0	1	1	0
Hepatocyte degenerative change	0	0	0	1	0	1	1	0	1	0	0	0
Hemopoiesis	0	0	0	1	0	1	2	1	1	1	0	1
Kupffer cell pigmentation	2	2	0	2	2	0	1	1	0	7	7	0
Leucocytosis	0	0	0	2	0	2	0	0	0	1	0	1
Microabscess(es)	0	0	0	0	0	0	0	0	0	0	0	0
Mineralization, focal	0	0	0	0	0	0	1	1	0	0	0	0
Necrosis, foci/areas	2	2	0	1	0	1	1	0	1	0	0	0
Necrosis, centrilobular	0	0	0	1	0	1	2	0	2	0	0	0
Necrosis, single-cell	1	0	1	3	2	1	3	2	1	3	3	0
Necrosis, periportal	0	0	0	0	0	0	0	0	0	1	0	1
Peliosis hepatis focal	0	0	0	0	0	0	1	0	1	0	0	0
Spongiosis hepatis	0	0	0	0	0	0	0	0	0	0	0	0
Thrombosis	0	0	0	1	0	1	0	0	0	1	0	1
Vacuolation foci/area(s)	0	0	0	2	2	0	0	0	0	0	0	0
Vacuolation centrilobular	0	0	0	1	0	1	0	0	0	0	0	0
Vacuolation single-cell	0	0	0	1	1	0	2	1	1	1	0	1
Vacuolation midzonal	0	0	0	0	0	0	0	0	0	0	0	0
Peritonitis	0	0	0	1	0	1	0	0	0	0	0	0
Adrenals, samples evaluated	50	39	11	50	38	12	50	39	11	50	49	9
Medullary hyperplasia	0	0	0	0	0	0	0	0	0	0	0	0
Invaded by malignant lymphoma	0	0	0	0	0	0	0	0	0	0	0	0
Angiectasis, cortex	9	8	1	14	11	3	12	10	2	12	8	4
Haemorrhagic degeneration, cortex	23	21	2	15	12	3	15	9	4	4	4	0
Congestion	2	0	2	0	0	0	1	0	1	1	0	1
Diffuse vacuolation	0	0	0	0	0	0	1	0	1	0	0	0
Focal vacuolation, cortex	1	1	0	1	1	0	1	1	0	2	2	0
Focal eosinophilic cellular change, cortex	0	0	0	0	0	0	0	0	0	0	0	0
Haemorrhage	0	0	0	0	0	0	0	0	0	0	0	0
Extramedullary hemopoiesis	0	0	0	0	0	0	2	0	2	1	0	1
Spleen, samples evaluated	50	39	11	50	38	12	50	39	11	50	41	9

Organ/Lesion	Females											
	Control			100 ppm			300 ppm			1000 ppm		
	T	TK	PD	T	TK	PD	T	TK	PD	T	TK	PD
Increased hemopoiesis	2	0	2	11	4	7	5	1	4	1	0	1
Increased hemosiderin	2	0	2	3	2	1	3	0	3	19	17	2
Myelofibrosis	0	0	0	0	0	0	0	0	0	1	1	0
Lymphoid depletion	0	0	0	1	0	1	0	0	0	0	0	0
Necrosis	0	0	0	0	0	0	0	0	0	0	0	0
Peritonitis	0	0	0	0	0	0	0	0	0	0	0	0

T =Total; TK = Terminal Kill, i.e. sacrificed at end of study; PD = Intercurrent death, i.e. found dead or killed in moribund state

Neoplastic findings

There was a slightly higher number of neoplasma in the males at a dose of 1000 ppm, due to a higher benign tumour count (Table 6.5-10.). The incidence of C-cell adenomas and carcinomas of the thyroid was increased in all treated male rats compared to controls (Table 6.5-12). This increase was mirrored by an incease in C-cell hyperplasia. There was, however, no clear dose-response relationship. Furthermore, the histopathology data revealed no evidence of progression from adenoma to carcinoma.

In addition, whilst the incidences of thyroid tumours observed in the study were well within the historical control data (HCD) provided, it is important to note that the HCD are combined from three sources.
1) 11 studies conducted at Bayer (1973 – 1976), Wistar (BOR:WISW(SPF-CPB)) from the same breeder, outside of the five year range of the study by B.6.5.1/01 and are therefore not relevant for comparison. HCD for Uterus adenocarcinoma was also from source 1 and therefore outside of five year range but included as supplementary information.
2) 25 studies (1981 – 1987) conducted at the same laboratory, Wistar (BOR:WISW(SPF-CPB)) from the same breeder. Considered the most relevant HCD for comparison.
3) data set from the Registry of Industrial Toxicology Animal-data (RITA) database; 29 studies, Wistar, breeder unknown. These data were not obtained at the same laboratory and are therefore not relevant for comparison.

HCD from Source 1, incidence of thyroid tumors:

Source 1	Interval (%)	
Years 1973-1976		
11 studies	males	females
C-cell adenoma	0.0-18.1	2.2-21.2
C-cell carcinoma	0.0-6.6	0.0-6.0
Follicular adenoma	0.0-5.3	0.0-2.4
Follicular carcinoma	0.0-1.5	0.0-1.1

HCD from source 2, incidence of thyroid tumors:

Source 2	**Interval (%)**	
Years 1981-1987		
25 studies	males	females
C-cell adenoma	0.0-17.0	0.0-14.3
C-cell carcinoma	0.0-16.0	0.0-4.3
Follicular adenoma	0.0-5.2	0.0-4.0
Follicular carcinoma	0.0	0.0

HCD from source 3, incidence of thyroid tumors:

Source 3	**Interval (%)**	
RITA		
29 studies	males	females
C-cell adenoma	2.0-16.0	0.0-17.3
C-cell carcinoma	0.0-3.0	0.0-4.1
Follicular adenoma	0.0-26.0	0.0-4.1
Follicular carcinoma	0.0-6.0	0.0-5.1

Overall, the C-cell tumours were within the most relevant HCD provided (source 2), and the increased incidence of C-cell tumours of the thyroid were considered unrelated to treatment due to the lack of a dose-response and lack of evidence of progression from adenoma to carcinoma. Follicular adenoma was slightly above the HCD range of HCD source 2 in high dose males and just within HCD range in high dose females but no progression to carcinomas were observed.

In female rats, a lower frequency of endometrial adenocarcinoma was found in comparison with controls (Table 6.5-12). These incidences were small and not dose-related. Overall, there were no treatment-related tumours of the uterus or of any other organ.

Table 6.5-10. Neoplastic histopathology results of the combined chronic toxicity/carcinogenicity study in rats (main groups)

Dose [ppm]	**Males**				**Females**			
	0	**100**	**300**	**1000**	**0**	**100**	**300**	**1000**
Number of animals examined	50	50	50	50	50	50	50	50
Mortality (%)	18	14	16	6	20	24	20	16
Body weight at termination [g]	396	404	413	387	253	264	259	248
Number of animals examined	50	50	50	50	50	50	50	50
Overall tumour incidence:	25	30	28	29	33	23	35	24
No. of animals with neoplasms	18	19	21	26	26	27	30	20
No. of animals with benign neoplasms	13	12	16	21	19	20	23	17
No. of animals with malignant neoplasms	6	9	5	6	11	10	7	4
No. of animals with multiple neoplasms	5	5	1	5	8	6	3	1

Table 6.5-11. Histopathology results of the combined chronic toxicity/carcinogenicity study in rats (main groups)

Organ\tissue tumour type		Dose [ppm]							
		0	100	300	1000	0	100	300	1000
		Males				Females			
Lung									
Examined	[N]	49	49	50	50	50	50	50	50
Carcinoma, alveolar/bronchiolar (m)	[N]	1	0	0	0	0	0	0	0
Liver									
Examined	[N]	49	49	50	50	49	50	50	50
Adenoma, hepatocellular (b)	[N]	0	0	0	0	0	1	3	0
Carcinoma, hepatocellular (m)	[N]	1	1	0	0	1	0	0	0
Stomach									
Examined	[N]	49	48	50	50	50	50	50	50
Papilloma (b)	[N]	0	0	1	0	0	0	0	0
Fibroma (b)	[N]	0	0	0	0	1	0	0	0
Leiomyosarcoma (m)	[N]	0	0	1	0	0	0	0	0
Ileum									
Examined	[N]	48	48	49	50	50	50	50	50
Leiomyoma (b)	[N]	0	0	0	0	0	1	0	0
Lymph Node (mesenteric)									
Examined	[N]	49	49	50	50	50	50	50	50
Haemangioma (b)	[N]	3	6	2	6	0	1	1	0
Pancreas									
Examined	[N]	49	48	50	50	49	50	50	50
Islet cell adenoma (b)	[N]	0	0	1	0	0	0	0	0
RHS									
Examined	[N]	50	49	50	50	50	50	50	50
Lymphoma (m)	[N]	2	1	2	2	0	0	0	0
Brain									
Examined	[N]	50	49	50	50	50	50	50	50
Granular cell tumour (b)	[N]	0	0	0	0	1	0	0	0
Granular cell tumour (m)	[N]	0	0	1	0	0	0	0	0
Pituitary									
Examined	[N]	50	49	50	50	50	50	49	50
Adenoma (b)	[N]	6	3	6	6	13	14	14	11
Adenocarcinoma (m)	[N]	1	0	0	0	0	0	2	1
Adrenals									
Examined	[N]	49	49	50	49	50	50	50	50
Ganglioneuroma (b)	[N]	0	0	0	0	0	1	0	0
Pheochromocytoma (b)	[N]	3	2	1	1	1	2	0	0
Pheochromocytoma (m)	[N]	1	2	1	0	1	0	0	0
Thymus									

Organ\tissue tumour type		Dose [ppm]							
		0	100	300	1000	0	100	300	1000
		Males				Females			
Examined	[N]	44	49	50	49	48	45	50	48
Thymoma (b)	[N]	0	0	0	0	0	1	0	0
Testicles									
Examined	[N]	49	49	50	50	-	-	-	-
Leydig cell tumour (b)	[N]	3	1	1	5	-	-	-	-
Epididymis									
Examined	[N]	49	49	50	50	-	-	-	-
Mesothelioma (m)	[N]	0	0	0	1	-	-	-	-
Stroma sarcoma (m)	[N]	1	0	0	0	-	-	-	-
Prostate									
Examined	[N]	49	49	50	50	-	-	-	-
Adenocarcinoma (m)	[N]	0	1	0	0	-	-	-	-
Preputial Gland #									
Adenocarcinoma (m)	[N]	-	1	-	-	-	-	-	-
Mammary Gland									
Examined	[N]	24	30	31	26	48	49	47	49
Fibroadenoma (b)	[N]	0	0	0	0	5	1	3	2
Adenocarcinoma (m)	[N]	0	0	0	0	2	2	2	1
Ovaries									
Examined	[N]	-	-	-	-	50	49	50	50
Granulosa cell tumour (b)	[N]	-	-	-	-	2	0	0	1
Theca cell tumour (b)	[N]	-	-	-	-	0	0	1	0
Theca cell tumour (m)	[N]	-	-	-	-	0	1	0	0
Granulosa theca cell tumour (m)	[N]	-	-	-	-	1	0	0	0
Uterus									
Examined	[N]	-	-	-	-	50	50	50	50
Adenoma (b)	[N]	-	-	-	-	0	0	1	0
Adenocarcinoma (m)	[N]	-	-	-	-	2	1	0	1
Leiomyosarcoma (m)	[N]	-	-	-	-	1	0	0	0
Stromal sarcoma (m)	[N]	-	-	-	-	2	2	1	0
Haemangioma (b)	[N]	-	-	-	-	1	0	1	0
Hemangiosarcoma (m)	[N]	-	-	-	-	0	1	0	0
Carcinoma, atypical (m)	[N]	-	-	-	-	0	3	2	1
Kidneys									
Examined	[N]	49	49	50	50	50	50	50	50
Transitional epithelium papilloma (b)	[N]	0	0	0	1	0	0	0	0
Liposarcoma (m)	[N]	0	0	0	1	0	0	0	0
Thyroid									

Organ\tissue tumour type		Dose [ppm]							
		0	**100**	**300**	**1000**	**0**	**100**	**300**	**1000**
		Males				**Females**			
Examined	[N]	50	49	50	50	49	50	50	50
Follicle adenoma (b)	[N]	0	1	0	3	0	0	1	2
C cell adenoma (b)	[N]	0	1	3	2	1	0	1	1
C cell carcinoma (m)	[N]	0	1	0	1	0	0	0	0
Parathyroid#									
Adenoma (b)	[N]	1	1	-	1	-	1	1	-
Skeletal Musculature									
Examined	[N]	49	49	50	50	50	50	50	50
Rhabdomyosarcoma (m)	[N]	0	0	0	0	0	1	0	0
Skin/Subcutis									
Examined	[N]	49	49	50	50	50	50	50	50
Keratoacanthoma (b)	[N]	0	1	0	0	0	0	0	0
Fibroma (b)	[N]	0	1	1	1	0	0	0	0
Basal cell carcinoma (m)	[N]	0	2	0	0	0	0	0	0
Epithelial tumour of skin adnexes (m)	[N]	0	0	0	0	1	0	0	0
Sarcoma, undifferentiated (m)	[N]	0	0	1	0	0	0	0	0
Bone									
Examined	[N]	49	49	50	50	50	50	50	50
Osteosarcoma (m)	[N]	0	0	0	1	0	0	0	0
Thoracic Cavity									
Examined	[N]	50	49	50	50	50	50	50	50
Sarcoma, undifferentiated (m)	[N]	0	0	0	0	1	0	0	0

(b) = benign neoplasms; (m) = malignant neoplasms

organ not routinely examined

Table 6.5-12. Thyroid and uterus tumour incidences

Dose [ppm]	Males					Females				
	0	**100**	**300**	**1000**	**HCD[b]**	**0**	**100**	**300**	**1000**	**HCD[b]**
Thyroid tumours										
Follicle adenoma (b)[a]	0/50	1/50	0/50	3/50	--	0/49	0/50	1/50	2/50	--
%	*0*	*2*	*0*	*6*	*0 – 5.2*	*0*	*0*	*2*	*4*	*0 - 4.0*
Follicle carcinoma	*0/49*	*0/49*	*0/50*	*0/50*		*0/50*	*0/50*	*0/50*	*0/50*	
C-cell hyperplasia	1/50	3/50	7/50	6/50	--	1/49	2/50	3/50	0/50	--
%	*2*	*6*	*14*	*12*	--	*2*	*4*	*6*	*0*	--
C-cell adenoma (b)[a]	0/50	1/49	3/50	2/50	--	1/49	0/50	1/50	1/50	--
%	*0*	*2*	*6*	*4*	*0 – 17.0*	*2*	*0*	*2*	*2*	*0 – 14.3*
C-cell carcinoma (b)[a]	0/50	1/49	0/50	1/50	--	0/49	0/50	0/50	0/50	--
%	*0*	*2*	*0*	*2*	*0 - 16.0*	*0*	*0*	*0*	*0*	*0 – 4.3*
Uterus tumours										
Adenoma (b) [a]						0/50	0/50	1/50	0/50	--

Dose [ppm]	Males					Females				
	0	**100**	**300**	**1000**	**HCD[b]**	**0**	**100**	**300**	**1000**	**HCD[b]**
Adenocarcinoma (m) [a]						2/50	1/50	0/50	1/50	
%						*4*	*2*	*0*	*2*	*0 - 14.4[c]*
Carcinoma; atypical (m) [a]						0/50	3/50	2/50	1/50	--
%						*0*	*6*	*4*	*2*	
Adenocarcinoma total [a]						1/50	4/50	2/50	2/50	
%						*4*	*8*	*4*	*4*	

[a] number of animals affected/total number of animals
[b] Combined historical control data from source 2. 25 studies (1981 – 1987), Wistar (BOR:WISW(SPF-CPB)) from the same breeder
[c] Combined historical control data from 11 studies conducted at Bayer (1973 – 1976), Wistar (BOR:WISW(SPF-CPB)) from the same breeder
(b) = benign neoplasms; (m) = malignant neoplasms

Conclusion
In a guideline dietary carcinogenicity study in rats, no carcinogenic effect was seen up to the top dose of 1000 ppm (equivalent to 55 mg/kg bw/day for males and 86 mg/kg bw/day for females respectively). Administration of tebuconazole to rats for two years caused effects on growth development in females at the top dose of 1000 ppm. Females appeared to be more sensitive than males. At the top dose (1000 ppm) the following effects were also seen in females: a treatment-related decrease in adrenal weight associated with a reduction in individuals with haemorrhagic degeneration of the cortex, increased spleen weight with associated haemosiderin accumulation and pigment deposits in the Kupffer star cells in the liver. No significant systemic toxicity was seen in males up to the top dose (55 mg/kg bw/day). The RMS notes that a higher dose should have been used in males to ensure a more robust result from the study.

Overall, a NOAEL of 1000 ppm (top dose) for males and females (equivalent to 55 mg/kg bw/day for males and 86 mg/kg bw/day for females respectively) was determined for carcinogenicity.

A NOAEL of 300 ppm for females (equivalent to 23 mg/kg bw/day in females) was determined for the systemic effect based on lower body weight gains and histopathological changes in the adrenal, spleen and liver in females at 1000 ppm. The systemic NOAEL for males was the top dose of 1000 ppm (55 mg/kg bw/day) as no significant systemic toxicity was seen in males. With the exception of the higher NOAEL for systemic toxicity in males, the other NOAELs are consistent with the original values agreed in the DAR (2006).

B.6.5.2. Studies in mice - combined chronic and carcinogenicity

Two studies are available.

a)

Previous evaluation	In DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.5.2/01
Study title	HWG 1608 – Study for cancerogenicity in NMRI mice (Administration in diet for up to twenty-one months); Addendum: Historical control data for hyper- and neoplastic liver findings in NMRI mice and the frequency of histiocytic sarcomas in NMRI-mice – A Compilation of historical data.
Dates (in life)	December 1984 – September 1986
Test substance	Tebuconazole
Purity (%) **Batch no.**	> 95 Mixed batch Fl. no.: 132 Single samples: 16001/84, PAV 994, 97.7%;16002/84, PAV 995, 95.6%; 16003/84, PAV 996, 95.8%; 16004/84, PAV 997, 96.2%; 16006/84, PAV 998, 98.3%
Test animals	Male and female-SPF-bred NMRI mice of the strain Bor:NMRI (SPF-Han) 5-6 weeks old and a mean weight of 29 g (24 g – 34 g) for males and 24 g (18 g – 31 g) for

	females
Groups	50/sex/group + satellite group 10/sex/group
Dose	0, 20, 60 or 180 ppm (corresponding to 0, 5.9, 18.2 or 53.1 mg/kg bw/day for males and 0, 9.0, 26.1 or 80.5 mg/kg bw/day for females)
Route	Oral, dietary
Vehicle	Wessalon (highly dispersed silicates), was added to the powdered food, to improve homogeneity and stability at a ratio of 1:1 (test compound: wessalon)
GLP	Yes
Guideline	OECD-Guideline 453 (1981) Note that the current guideline was adopted in 2009.
Deviation	The following deviations from the current OECD-Guideline 453 (2009) occurred: - Haematological examination time points only after 12 and 21 months and prothrombin time and activated partial thromboplastin time was not examined. - Serum electrolytes, glucose, triglycerides, albumin were not determined and the quantity of clinical chemistry examinations were not sufficient. - Urinalyses was not conducted - Weight of the following organs was not determined: epididymides, ovaries, thyroid (incl. parathyroids) and uterus - The following tissues were not subject to histopathological examination: coagulating gland, lacrimal gland, parathyroid gland.
Impact of deviations	Minor – the deviations are minimal, can be compensated by the results of other studies and thus they are not considered to affect the validity of the study.
Acceptable	Acceptable
NOAEL	Carcinogenicity: 180 ppm (the top dose) for males and females (equivalent to 53 and 81 mg/kg bw/day for males and females respectively). Systemic toxicity: 20 ppm for males and females (equivalent to 6 and 9 mg/kg bw/day for males and females respectively).
Effects at the LOAEL	Carcinogenicity: based on the absence of treatment-related neoplastic effects in treated animals. Systemic toxicity: based on fatty degeneration/vacuolation of the liver in both sexes and increases in bilirubin levels in females at 60 ppm (equivalent to 18 and 26 mg/kg bw/day for males and females respectively).

Methods

Groups of 50 male and 50 female NMRI mice were given tebuconazole at concentrations of 0, 20, 60 and 180 ppm for 21 months in the diet (groups 1 - 8). Groups of ten similarly treated male and female animals (satellite groups, groups 9 - 16) were sacrificed after a period of 12 months. The tebuconazole doses were based on the results of two previous feeding studies lasting four and eight weeks respectively, in NMRI-mice of the same strain (Ramm & Karbe, 1986; and Ramm & Schilde, 1986) (B.6.3.1.2). Tebuconazole was administered to the animals in the treatment groups from start of study until spontaneous death or time of sacrifice, via *ad libitum* consumption in the diet.

The animals were inspected at least twice daily, and any clinical signs and special features were noted. Detailed individual inspections took place once a week. Body surfaces, orifices, posture, general behaviour, respiration and excretory products were assessed. Individual body weight was recorded weekly for the first 13 weeks and once every two weeks thereafter. Food and water intake were determined group-wise from start of the study up to and including week 13 once a week, and from week 15 every two weeks. Laboratory examinations of blood were made of ten animals per group after 12 and 21 months. Animals which died spontaneously during the study or were moribund and sacrificed were dissected and their organs/tissue subjected to detailed gross pathological examination.

Table 6.5-13. Study design and dose received

Test group		**1**	**2**	**3**	**4**
Concentration in diet	(ppm)	**0**	**20**	**60**	**180**
Dose per animal [mg/kg bw/day]	Male	0	5.9	18.2	53.1
	Female	0	9.0	26.1	80.5

Results

Clinical observations

Administration of the test item had no effect on mortality (Table 6.5-14) or overt clinical signs of toxicity at any tested dose. No abnormalities were noted in body surfaces and orifices, general behaviour, posture, respiration, excretory products and eyes of treated animals. The incidence, location and chronology of palpable tissue masses did not indicate any treatment-related effect. An increase in the frequency of bristled coats was observed in males at all doses when compared to control animals. In females this finding occurred at the same frequency in controls and treated animals. As this clinical finding, in males only, occurred in isolation, it was not considered treatment-related. Overall, there were no adverse, treatment-related effects on mortality or clinical signs of toxicity.

Table 6.5-14. Mortality results

	Dose [ppm]							
	0	**20**	**60**	**180**	**0**	**20**	**60**	**180**
	Males				**Females**			
Number of animals	50	50	50	50	50	50	50	50
Week 1-13	1	0	0	0	0	1	0	0
[%]	2	0	0	0	0	2	0	0
Week 1-26	1	0	2	0	1	2	1	0
[%]	2	0	4	0	2	4	2	0
Week 1-52	1	0	4	0	5	4	3	4
[%]	2	0	8	0	10	8	6	8
Week 1-78	5	10	13	12	18	18	13	23
[%]	10	20	26	24	36	36	26	46
Week 1-91	12	22	21	22	33	28	27	32
[%]	24	44	42	44	66	56	54	64
Week 1-93	12	22	21	22	33	29	27	32
[%]	24	44	42	44	66	58	54	64

Body weight and food intake

Main groups: Lower body weights were observed in males in all treated groups compared to controls; this was observed throughout weeks 4 - 92 (Table 6.5-15). This was seen mainly during the first eight weeks and with some statistical significance. Male body weights in the 180 ppm dose group were at times significantly lower (between the 13th and 31st study weeks). However, due to the small differences from controls and absence of a dose-correlation, these effects are not considered treatment-related or toxicologically relevant. Male and female mice in all dose groups consumed about the same amount of food and water as the corresponding controls (Tables 6.5-13 and 6.5-14).

Satellite groups: No body weight effects were seen.

Overall, there were no adverse, treatment-related effects on body weight and food intake.

Table 6.5-15. Body weight development

	Body Weight – Mean (g)							
	Weeks							
Dose [ppm]	**0**	**4**	**8**	**13**	**25**	**53**	**79**	**92**
	Males							
0	29	36	39	41	46	49	48	46
20	29	34**	37*	40	45	49	48	47
60	29	35*	37	40	45	48	48	46
180	29	35	37*	39**	45	48	47	44
	Weeks							
	0	**4**	**8**	**13**	**25**	**53**	**79**	**91**

	Females							
0	24	26	28	30	33	37	38	39
20	24	26	28	30	33	39*	40	40
60	24	27*	28	31*	34	38	40	39
180	25	26**	28	31*	33	38	39	41

* p<0.05; **p<0.01

Table 6.5-16. Food intake

Dose [ppm]	Food intake – Main groups (group 1-8, 21 months)			
	g/animal		g/kg body weight	
	Total[#]	Per day	Total[#]	Per day
Males				
0	8053	12.6	182503	286.5
20	8145	12.8	187667	294.6
60	8448	13.3	193364	303.6
180	8055	12.6	188021	295.2
Females				
0	9425	14.8	283007	444.3
20	9752	15.3	286054	449.1
60	9524	15.0	276949	434.8
180	9660	15.2	285013	447.4

[#] total intake in 637 days

Table 6.5-17. Water intake

Dose [ppm]	Water intake – Main groups (group 1-8, 21 months)			
	g/animal		g/kg body weight	
	Total[#]	Per day	Total[#]	Per day
Males				
0	5430	8.5	121751	190.2
20	5669	8.9	129135	201.8
60	5436	8.5	123440	192.9
180	5307	8.3	123079	192.3
Females				
0	6595	10.3	193744	302.7
20	6631	10.4	191180	298.7
60	6384	10.0	182389	285.0
180	6872	10.7	198734	310.5

[#] total intake in 640 days

Haematology and clinical chemistry

No effects on haematology up to and including 60 ppm were seen. At a dose of 180 ppm (the top dose), temporary (at 51st week in the satellite groups) and statistically significantly different erythrocyte counts (increased in males and reduced in females), haemoglobin and hematocrit values (reduced in females only) were seen. However, in females these findings no longer existed after 90 weeks; therefore they were not regarded as toxicologically-relevant. At this dose (180 ppm), male mice showed statistically significantly lower erythrocyte counts at the end of study (Table 6.5-18.). Overall, therefore, only the lower erythrocyte counts in males at the top dose were considered treatment-related and adverse.

The thrombocyte count for males in all the treatment groups showed statistically significantly lower values compared to control males at 51st week (Table 6.5-18.). However the male control figures at this time were

unusually high, while the figures of the treated animals were within the normal variation range. Additionally, since there were no significant differences from the controls in the 90th week, this finding is regarded as not toxicologically-relevant.

Table 6.5-18. Haematology data from the 51st week and the 90th week in mice treated with tebuconazole in their diet through 21 months

Haematology										
Dose [ppm]	**Week**	**LEUCO [10^9/L]**	**ERY [10^{12}/L]**	**HB [g/L]**	**HCT [L/L]**	**MCV [fL]**	**MCH [pg]**	**MCHC [g/L ERY]**	**THRO [10^9/L]**	**RETI [‰]**
Males										
0	51	5.4	8.30	137	0.46	56	16.5	295	1585	19
20	51	4.5	8.57	140	0.48	56	16.4	294	**1350***	17
60	51	4.5	8.41	136	0.47	55	16.2	293	**1351***	18
180	51	5.2	**8.73* (+5.2%)**	140	0.49	56	16.0	**288***	**1277****	**15****
Females										
0	51	4.7	8.30	142	0.47	57	17.1	301	840	22
20	51	4.7	8.07	140	0.46	57	17.4	303	838	23
60	51	5.7	8.42	141	0.47	56	16.8	299	854	18
180	51	4.2	**7.88* (-5.1%)**	**133** (-6.3 %)**	**0.45* (-4.3 %)**	57	16.9	299	921	20
Males										
0	90	7.1	8.20	144	0.428	52	17.5	336	1478	18
20	90	7.0	8.55	154	0.432	51	18.1	**356***	1432	20
60	90	6.8	8.15	145	0.426	52	17.8	340	1451	18
180	90	8.8	**7.65** (-6.7%)**	139	0.410	54	18.1	339	1445	12
Females										
0	90	6.2	7.87	143	0.409	52	18.2	350	975	23
20	90	5.1	**7.26***	135	0.390	54	18.6	346	975	21
60	90	5.8	7.13	131	0.393	56	18.4	**333***	1145	23
180	90	7.2	7.56	141	0.392	52	18.5	361	998	25

* significantly different from control $p \leq 0.05$
** significantly different from control $p \leq 0.01$
Statistically significant values are written in **bold letters**.

Table 6.5-19. Differential blood count data in mice treated with tebuconazole in their diet through 21 months

Differential blood count [%]						
Dose [ppm]	**Week**	**EOSIN**	**STAB**	**SEGM**	**LYM**	**MONO**
Males						
0	51	0.0	0.0	16.3	83.4	0.3
20	51	0.2	0.2	20.7	78.6	0.3
60	51	0.2	0.0	21.1	77.7	1.0
180	51	0.3	0.2	17.7	81.3	0.5
Females						
0	51	0.3	0.1	13.7	84.7	1.2

Differential blood count [%]						
Dose [ppm]	**Week**	**EOSIN**	**STAB**	**SEGM**	**LYM**	**MONO**
20	51	0.1	0.0	10.9	88.3	0.7
60	51	0.6	0.2	13.6	84.2	1.4
180	51	0.1	0.2	11.7	87.4	0.6
Males						
0	90	0.2	0.3	20.3	77.7	1.5
20	90	0.1	0.3	22.9	76.0	0.7
60	90	0.1	0.1	23.9	73.6	2.3
180	90	0.2	0.1	**35.1***	**61.6***	3.0
Females						
0	90	0.8	0.8	28.9	67.0	2.5
20	90	0.8	0.7	24.8	70.1	3.5
60	90	0.3	0.4	31.6	63.4	4.3
180	90	0.2	0.2	18.1	**79.7***	1.8

* statistically significant difference from control p≤0.05
** statistically significant difference from control p≤0.01
Statistically significant values are written in **bold letters**.

The total bilirubin concentration was statistically significantly increased (with a clear dose-response) in the 53rd week for the females in the 180 ppm dose group, and in the 92nd week, also in females, from 20 ppm and above (Table 6.5-20.). The increase at 20 ppm (in the 92nd week) was small and resulted in a value (2.6 µmol/L) which was even lower than the control value at 53rd week (2.7 µmol/L). Overall, therefore, there was a treatment-related and adverse increase in total bilirubin levels in females from 60 ppm.

In addition males and females in the 180 ppm group showed statistically significant and distinctly lower cholesterol concentrations in the plasma in the 53rd week. At end of study the males' figures in this group (180 ppm) were still low (but not statistically significantly); the females' however were not still lower compared to controls. The lower cholesterol values for the 60 ppm dose group females at end of study should be regarded, in the absence of a dose-response, as not treatment-related. Overall, treatment-related decreases in cholesterol were seen in males and females at the top dose (180 ppm).

Table 6.5-20. Clinical chemistry data in mice treated with tebuconazole in their diet through 21 months in 53rd week and the 92nd week (% change compared with control)

Clinical chemistry (blood)									
Dose [ppm]	**Week**	**ASAT (GOT) [U/L]**	**ALAT (GPT) [U/L]**	**APh [U/L]**	**t-BILI [µmol/L]**	**PROT [g/L]**	**HST UREA [mmol/L]**	**CHOL [mmol/L]**	**CREA [µmol/L]**
Males									
0	53	31.5	35.4	93	3.9	59.2	10.56	4.66	41
20	53	28.3	30.3	95	3.8	59.2	11.13	4.38	35
60	53	30.5	36.4	84	3.8	57.1	9.65	4.36	36
180	53	33.6	45.8	107	3.9	58.4	9.75	**3.61* (-22.5%)**	34
Females									
0	53	59.3	59.1	196	2.7	58.2	8.98	3.86	39
20	53	34.7	**32.8****	260	3.2	58.6	9.40	4.32	40
60	53	42.5	44.0	180	3.3 (+22.2%)	59.2	9.52	3.43	41
180	53	41.6	53.3	186	**3.7** (+37%)**	57.6	9.90	**2.44** (-36.8%)**	42
Males									

Clinical chemistry (blood)									
Dose [ppm]	**Week**	**ASAT (GOT) [U/L]**	**ALAT (GPT) [U/L]**	**APh [U/L]**	**t-BILI [µmol/L]**	**PROT [g/L]**	**HST UREA [mmol/L]**	**CHOL [mmol/L]**	**CREA [µmol/L]**
0	92	49.1	74.3	152	3.3	65.0	8.43	4.31	29
20	92	40.4	56.6	141	3.2	63.3	7.76	3.93	36
60	92	42.9	49.4	131	3.3	60.4	8.14	3.93	32
180	92	53.1	83.4	153	3.4	61.2	8.01	3.27 (-24.1%)	36
Females									
0	92	60.9	72.9	168	2.2	56.0	9.45	3.57	26
20	92	52.2	66.0	**393***	**2.6* (+9.6%)**	59.8	8.35	3.53	26
60	92	72.0	73.8	201	**3.4** (+54.5%)**	58.1	8.82	**2.97***	**32***
180	92	71.7	101.4	227	**3.6** (+63.6%)**	**60.6***	8.77	3.46	**36****

* statistically significant difference from control p≤0.05

** statistically significant difference from control p≤0.01

Statistically significant values are written in **bold letters**.

Gross pathology

The gross pathological findings recorded on autopsy at end of the study did not provide any indications of treatment-related effects. The majority of animals exhibited the typical spontaneous alterations in organs and tissues for animals of this age.

Organ Weights

The absolute (Table 6.5-21.) and relative (Table 6.5-22.) liver weights were increased compared to the controls, in male and female mice at 180 ppm, both at the interim autopsy and the final autopsy. However, while a dose-response was seen, statistical significance was only noted in the male relative liver weights at end of study. Overall, treatment-related increases in liver weight were seen at the top dose.

Table 6.5-21. Absolute organ weight data in mice treated with tebuconazole in their diet through 21 months

Organ weights, absolute [mg]									
Dose [ppm]	**Week**	**Brain**	**Heart**	**Lungs**	**Liver**	**Spleen**	**Kidneys**	**Adrenals**	**Testes**
Males									
0	53	501	308	284	2421	163	853	7	276
20	53	478	**253****	289	2148	172	**733***	8	258
60	53	487	297	**266***	2332	147	843	6	267
180	53	498	269	267	2461	180	803	9	257
Females									
0	53	502	209	233	1817	202	504	16	-
20	53	507	186	218	1665	219	489	14	-
60	53	510	204	230	1900	201	524	16	-
180	53	497	219	262	1980	212	482	17	-
Males									
0	92/93	495	288	299	2294	167	865	9	239
20	92/93	513	292	296	2281	167	839	13	221
60	92/93	500	277	290	2325	178	829	9	237
180	92/93	500	287	289	2423	142	858	9	225

Organ weights, absolute [mg]									
Dose [ppm]	**Week**	**Brain**	**Heart**	**Lungs**	**Liver**	**Spleen**	**Kidneys**	**Adrenals**	**Testes**
Females									
0	92/93	504	206	250	2255	250	533	13	-
20	92/93	504	214	286	2131	285	548	14	-
60	92/93	504	211	293	2284	249	539	12	-
180	92/93	509	221	273	2822	269	574	14	-

* significantly different from control $p \leq 0.05$
** significantly different from control $p \leq 0.01$
Statistically significant values are written in **bold letters**.

Table 6.5-22. Relative organ weight data in mice treated with tebuconazole in their diet through 21 months

Organ weights, relative [mg/100 g]										
Dose [ppm]	**Week**	**Body Weight [g]**	**Brain**	**Heart**	**Lungs**	**Liver**	**Spleen**	**Kidneys**	**Adrenals**	**Testes**
Males										
0	53	49	1044	638	590	4987	335	1763	14	569
20	53	45	1073	565	646	4736	**381***	1623	17	573
60	53	48	1018	623	554	4809	307	1744	12	560
180	53	48	1060	567	567	5123	370	1704	19	544
Females										
0	53	37	1382	572	634	4932	549	1388	43	-
20	53	35	1472	532	633	4750	630	1398	41	-
60	53	**40***	1272	510	576	4746	501	1304	41	-
180	53	38	1330	582	700	5260	562	1284	45	-
Males										
0	92/93	46	1072	625	645	4943	359	1850	20	518
20	92/93	47	1120	631	650	4908	364	1799	29	478
60	92/93	47	1076	592	**623***	4970	385	1767	19	508
180	92/93	46	1102	633	636	**5287****	311	1897	21	496
Females										
0	92/93	39	1290	523	638	5686	626	1355	34	-
20	92/93	40	1263	535	714	5308	683	1366	35	-
60	92/93	39	1306	546	756	5804	639	1389	31	-
180	92/93	41	1263	546	674	6902	649	1412	36	-

* significantly different from control $p \leq 0.05$
** significantly different from control $p \leq 0.01$
Statistically significant values are written in **bold letters**.

Non-neoplastic alterations
Non-neoplastic histopathological findings were only seen in the liver.
Satellite groups: In the satellite groups, four of ten males in the 180 ppm dose group, six females in the 180 ppm dose group, and five females in the 60 ppm dose group exhibited a mostly minimal periportal vacuolization in the liver. These vacuoles were shown to be lipid-containing by the Oil Red O stain. The type, number and distribution

of the other findings did not indicate a treatment-related effect.

Table 6.5-23. Non neoplastic histopatological findings in the liver at interim autopsy

	males				**females**			
	0	20	60	180	0	20	60	180
	10	10	10	10	10	10	10	10
Minimal periportal fine vacuolation							1	
Minimal focal periportal vacuolation				4			4	5
Moderate periportal vacuolation								1
Minimal centrilobular fine vacuolation				1			1	2
Minimal focal centrilobular fine vacuolation	5	2	1	1			1	2
Minimal centrilobular vacuolation	4	1						

Main groups: The histopathological examination of the livers in the main groups revealed an increased number of animals with periportal vacuolization of the liver at 180 ppm which was only marginal in the females. In addition, the number of animals with centrilobular fatty vacuolization in the males at 60 and 180 ppm was higher than in the control group. The examination of the other organs and tissue in the main groups did not provide any indications of non-neoplastic alterations which might be attributed to treatment. Overall, fatty vacuolation/degeneration of the liver was seen in both sexes from 60 ppm; this was considered adverse and treatment-related.

Table 6.5-24. Non neoplastic histopatological findings in the liver at terminal autopsy

	males				**females**			
Dose (ppm)	0	20	60	180	0	20	60	180
No of animals examined	50	50	50	50	50	50	50	50
Minimal focal periportal fine vacuolation							1	
Minimal periportal fine vacuolation				1	1	4	1	4
Marked periportal fine vacuolation					1			
Minimal focal periportal vacuolation			1	8			1	2
Minimal periportal vacuolation						1	1	1
Moderate periportal vacuolation							1	1
total	**0**	**0**	**1**	**9**	**2**	**5**	**5**	**8**
Minimal focal centrilobular fine vacuolation		2	5	2	1		1	
Minimal centrilobular fine vacuolation		1	3	8	2	1	1	3
Moderate centrilobular fine vacuolation			1	4	1		1	1
Marked centrilobular fine vacuolation				1				
Minimal focal centrilobular vacuolation	3	3		2				1
Minimal centrilobular vacuolation	2	2			2	4	1	2
Moderate focal centrilobular vacuolation					1			
Moderate centrilobular vacuolation			1				2	2
Marked centrilobular vacuolation							1	
Total	**5**	**8**	**10**	**17**	**7**	**5**	**7**	**9**

Neoplastic changes
No treatment-related increases in overall tumour incidence were seen in animals of the satellite and the main groups (Tables 6.5-23 to 6.5-25.).

Table 6.5-25. Number male and female mice with benign and/or malignant tumours (interim autopsy)

Dose [ppm]		**0**	**20**	**60**	**180**
			Males		
Mice examined	[N]	10	10	10	10
Tumour host	[N]	2	1	2	1
Solely benign tumours	[N]	2	1	1	0
Solely malignant tumours	[N]	0	0	1	1
			Females		
Mice examined	[N]	10	10	10	10
Tumour host	[N]	2	2	1	1
Solely benign tumours	[N]	2	1	1	1
Solely malignant tumours	[N]	0	1	0	0

Table 6.5-26. Listing of all tumours in respect to number, location, type and dignity (interim autopsy)

Organ/Tissue Tumour Type		Dose [ppm]							
		0	20	60	180	0	20	60	180
		Males				Females			
RHS									
Examined	[N]	10	10	10	10	10	10	10	10
Lymphosarcoma (m)	[N]	0	0	0	0	0	1	0	0
Lung									
Examined	[N]	10	10	10	10	10	10	10	10
Adenoma (b)	[N]	2	1	1	0	2	0	1	1
Adenocarcinoma (b)	[N]	0	0	1	0	0	0	0	0
Uterus									
Examined	[N]	-	-	-	-	10	10	10	10
Leiomyoma (b)	[N]	-	-	-	-	0	1	0	0
Femur									
Examined	[N]	10	10	10	10	10	10	10	10
Rhabdomyosarcoma (m)	[N]	0	0	0	1	0	0	0	0

(b) = benign neoplasms; (m) = malignant neoplasms

Table 6.5-27. Number male and female mice with benign and/or malignant tumours (main group)

Dose [ppm]		0	20	60	180
		Males			
Mice examined	[N]	50	50	50	50
Tumour host	[N]	31	36	32	26
Solely benign tumours	[N]	20	17	17	14
Solely malignant tumours	[N]	6	12	9	10
Benign and malignant tumours	[N]	5	7	6	2
		Females			
Mice examined	[N]	50	50	50	50
Tumour host	[N]	25	33	35	25
Solely benign tumours	[N]	6	8	10	11
Solely malignant tumours	[N]	15	15	19	10
Benign and malignant tumours	[N]	4	10	6	4

Table 6.5-28. Listing of all tumours with number, location, type and dignity

Organ\tissue tumour type		Dose [ppm]							
		0	20	60	180	0	20	60	180
		Males				Females			
RHS									
Number examined	[N]	50	49	50	49	49	49	50	50
Lymphosarcoma (m)	[N]	4	5	2	3	9	11	11	5
Pleomorph Lymphosarcoma (m)	[N]	0	0	0	0	0	3	1	0
Lymphoid leukaemia (m)	[N]	0	1	2	0	5	5	4	2
Myeloid leukaemia (m)	[N]	0	0	0	0	0	1	0	0
Histiocytary sarcoma (m)	[N]	0	1	2	1	1	0	0	0
Lung									

Organ\tissue tumour type		Dose [ppm]							
		0	**20**	**60**	**180**	**0**	**20**	**60**	**180**
		Males				**Females**			
Number examined	[N]	50	49	50	49	49	49	50	50
Adenoma singular (b)	[N]	10	6	7	7	4	10	6	6
Adenoma multiple (b)	[N]	4	5	4	0	0	3	1	1
Adenocarcinoma singular (m)	[N]	5	6	7	4	0	5	3	1
Adenocarcinoma multiple (m)	[N]	0	2	0	0	0	0	3	1
Liver									
Number examined	[N]	50	49	50	49	49	49	50	50
Hepatocellular tumour singular (b)	[N]	2	2	4	6	1	0	0	0
Hepatocellular tumour multiple (b)	[N]	0	0	1	0	0	0	0	0
Hepatocellular adenoma singular or multiple	[N]	2 (4 %)	2 (4 %)	5 (10 %)	6 (12%)	1	0	0	0
Hepatocellular carcinoma	[N]	1	0	0	1	0	0	0	1
Haemangioma (b)	[N]	1	2	0	0	1	0	0	0
Hemangiosarcoma (m)	[N]	0	0	0	1	0	0	0	0
Spleen									
Number examined	[N]	50	49	50	49	49	49	50	50
Haemangioma (b)	[N]	0	0	0	0	0	0	0	1
Hemangiosarcoma (m)	[N]	0	0	0	1	1	0	0	0
Pancreas									
Number examined	[N]	50	49	49	48	48	48	50	50
Islet cell adenoma (b)	[N]	0	1	0	0	0	0	0	0
Urinary bladder									
Number examined	[N]	50	49	50	49	49	49	50	49
Leiomyosarcoma (m)	[N]	0	0	0	1	0	0	0	0
Fibrosarcoma (m)	[N]	0	1	0	0	0	0	0	0
Uterus									
Number examined	[N]	-	-	-	-	49	49	50	50
Endometrial sarcoma (m)	[N]	-	-	-	-	0	1	0	0
Deciduoma (b)	[N]	-	-	-	-	0	1	0	0
Leiomyosarcoma (m)	[N]	-	-	-	-	0	1	0	0
Haemangioma (b)	[N]	-	-	-	-	0	0	0	1
Ovaries									
Number examined	[N]	-	-	-	-	49	49	50	5
Cystadenoma (b)	[N]	-	-	-	-	0	0	0	1
Papillary cystadenoma (b)	[N]	-	-	-	-	0	1	1	0
Tubular adenoma (b)	[N]	-	-	-	-	2	1	2	0
Sertoliform tubular adenoma (b)	[N]	-	-	-	-	0	1	0	0
Bilateral tubular adenoma (b)	[N]	-	-	-	-	0	0	0	1
Luteoma	[N]	-	-	-	-	1	2	0	2
Granulosa cell tumour (b)	[N]	-	-	-	-	2	2	3	1

Organ\tissue tumour type		Dose [ppm]							
		0	**20**	**60**	**180**	**0**	**20**	**60**	**180**
		Males				**Females**			
Granulosa cell tumour (m)	[N]	-	-	-	-	0	0	1	0
Prostate									
Number examined	[N]	47	47	50	48	-	-	-	-
Carcinoma (m)	[N]	1	0	0	0	-	-	-	-
Seminal vesicle									
Number examined	[N]	49	49	50	49	-	-	-	-
Carcinoma (m)	[N]	0	0	1	0	-	-	-	-
Testicles									
Number examined	[N]	50	49	50	49	-	-	-	-
Leydig cell tumour (b)	[N]	0	1	0	0	-	-	-	-
Epididymis									
Number examined	[N]	50	49	50	49	-	-	-	-
Anaplastic sarcoma (m)	[N]	0	1	0	0	-	-	-	-
Fibrosarcoma (m)	[N]	1	0	0	0	-	-	-	-
Thyroid									
Number examined	[N]	50	49	50	50	48	50	49	50
Follicular adenoma (b)	[N]	0	1	0	1	0	1	0	0
Adrenals									
Number examined	[N]	49	49	50	49	49	49	50	49
Cortical adenoma singular (b)	[N]	6	10	6	2	0	0	0	0
Cortical adenoma multiple (b)	[N]	2	2	4	0	0	0	0	0
Pheochromocytoma (b)	[N]	0	0	0	1	0	0	0	1
Pituitary									
Number examined	[N]	49	48	48	49	45	47	45	44
Adenoma (b)	[N]	1	0	0	0	0	1	4	2
Schwannoma (m)	[N]	0	1	0	0	0	0	0	0
Skeletal musculature									
Number examined	[N]	50	49	50	49	49	49	49	50
Rhabdomyosarcoma (m)	[N]	0	0	0	0	0	0	0	1
Skin									
Number examined	[N]	49	50	50	50	49	49	50	50
Squamous epithelial carcinoma (m)	[N]	0	0	0	0	0	0	0	1
Brain									
Number examined	[N]	50	49	50	50	50	50	49	50
Meningioma (m)	[N]	0	1	0	0	0	0	0	0
Harder's glands									
Number examined	[N]	49	50	49	50	49	49	50	50
Adenoma (b)	[N]	3	2	4	3	1	1	0	0
Ear scoops[#]									
Fibrosarcoma (m)	[N]	1	0	0	0	0	0	0	0
Subcutaneous tissue[#]									
Fibrosarcoma (m)	[N]	0	1	0	0	0	0	0	0

Organ\tissue tumour type		Dose [ppm]							
		0	**20**	**60**	**180**	**0**	**20**	**60**	**180**
		Males				**Females**			
Cornifying basal cell carcinoma(m)	[N]	0	0	0	0	0	0	0	1
Mammary Gland									
Number examined	[N]	50	49	50	49	49	49	50	50
Adenocarcinoma (m)	[N]	0	0	0	0	3	1	3	2
Thoracic cavity#									
Fibrosarcoma (m)	[N]	0	0	1	0	0	0	0	0
Head#									
Haemangioma (b)	[N]	0	0	0	0	0	0	1	0
Cervix/Vagina									
Number examined	[N]	-	-	-	-	48	48	50	50
Cervical adenocarcinoma (m)	[N]	-	-	-	-	0	1	0	0
Vaginal leiomyoma (b)	[N]	-	-	-	-	1	0	1	0
Primary location unknown squamous epithelial									
Carcinoma	[N]	0	0	0	1	0	0	0	0

(b) = benign neoplasms; (m) = malignant neoplasms
organ not routinely examined

The number of hepatocellular adenoma in males in the 60 ppm and 180 ppm dose groups was slightly higher compared with the control and low dose group (20 ppm) (Table 5.6-26). The incidence of hepatocellular adenoma in males was 4 % / 4 % / 10 % / 12 % at 0, 20, 60 and 180 ppm respectively. In the male high dose group (180 ppm) and in the control group there was also one animal each with a carcinoma.

HCD for benign and malignant liver tumours were provided in the report; these ranged from 1/46 (2 %) to 9/50 (18 %) (Table 6.5-27). These HCD were obtained within 5 years of the study date, using the same species, strain and breeder as the study. However, no information on the laboratory was provided and more importantly, no distinction between malignant and benign, singular and multiple tumour incidences was presented; therefore, these HCD should not be compared directly with the increased incidences of liver adenoma seen in males in this study. HCD (2 % - 16 %) for liver adenoma in males in the same strain of mice and relevant period (1984 - 1996) from the Registry of Industrial Toxicology Animal-data (RITA) database have also been provided (Table 6.5-28); although these data were not obtained from the same laboratory where the study was conducted, they clearly show that the increased incidences (10/12 %) of liver adenoma seen in males in this study were clearly within the range of the RITA HCD. Overall, therefore, it can be concluded that there were no treatment-related tumours in this study up to the top dose of 180 ppm.

Table 6.5-29. Incidence of hepatocellular tumours in historical studies

Study Number	**1**	**2**	**3**	**4**	**5**	**6**
Liver						
Number examined	50	50	50	45	46	48
Hepatocellular tumours (b+m)	7	3	9	5	1	6
[%]	14	6	18	11	2	12

Study Number	**1**	**2**	**3**	**4**	**5**	**6**

(b) = benign neoplasms; (m) = malignant neoplasms

Details of HCD presented:

Years conducted – 1981 – 1988 (Note: within 5 years of the study date 1984 – 1986)

Laboratory – information not provided for the HCD

Species – Mouse (Note: same as used in the study)

Strain – NMRI (Note: same as used in the study)

Breeder –Same as used in the study

Table 6.5-30. Hepatocellular tumours in mice, historical RITA data

Mice strain	**Studies (N)**	**Dates of study**	**Duration (months)**	**Number of animals**	**Adenoma, hepatocellular**		**Carcinoma, hepatocellular**	
					with lesion	**in %**	**with lesion**	**in %**
Male								
NMRI	6	1984 – 1996	19 – 24	348	27	2.0 – 16.0 mean 7.8	21	0.0 – 20.0 mean 6.0
All*	113	1984 - 2013	6 - 25	5167	391	0.0 – 22.0 mean 7.6	459	0.0 – 22.0 mean 8.9
Female								
NMRI	6	1984 – 1996	19 – 24	210	3	0.0 – 2.0 mean 1.4	4	0.0 – 4.0 mean 1.9
All*	113	1984 - 2013	6 - 25	5112	74	0.0 – 13.3 mean 1.4	57	0.0 – 12.2 mean 1.1

*the studies conducted over 6 months were in RasH2 and p53 which had very low incidences in liver tumours. Thus, considering only classical carcinogenicity studies conducted over 19 – 25 months, mean incidences are higher.

Conclusion

In conclusion, in a guideline chronic toxicity/carcinogenicity assay, administration of tebuconazole to mice for 21 months was well tolerated without adverse effects at doses up to and including 20 ppm. At a dose of 60 ppm and above slight fatty degeneration/vacuolation of the liver was seen in both sexes and an increase in bilirubin was seen in females. At the top dose of 180 ppm there were also increases in absolute and relative liver weights (statistically significant for males only), decreases in cholesterol in both sexes and reductions in erythrocyte counts in males. No treatment-related tumour findings were observed up to the top dose of 180 ppm.

Overall, a NOAEL of 180 ppm (the top dose) for males and females (equivalent to 53 and 81 mg/kg bw/day for males and females respectively) was determined for carcinogenicity, a dose at which liver toxicity and changes in some clinical-chemistry and haematological parameters occurred. This NOAEL is consistent with the original value agreed in the DAR (2006).

A NOAEL of 20 ppm for males and females (equivalent to 6 and 9 mg/kg bw/day for males and females respectively) was determined for systemic effects based on liver toxicity (fatty degeneration/vacuolation) in both sexes and increases in bilirubin in females at 60 ppm (18 and 26 mg/kg bw/day for males and females respectively) . This NOAEL has been amended from the original value agreed in the DAR (2006); the original systemic NOAEL for females (60 ppm) has been reduced to 20 ppm, becoming equivalent to that set originally for males.

b)

Previous evaluation	In DAR (2006) for original approval

Study ID	B.6.5.2/02
Study title	HWG 1608 – Toxic dose-range carcinogenicity study in NMRI mice (Supplement to study T 6018953 (= Report no. 16376) with administration in diet over a 21-month period)
Dates (in life)	22 August 1988 – 25 May 1990

Test substance	Tebuconazole
Purity (%) **Batch no.**	96.2 816896061
Test animals	Male and female NMRI mice; 5-6 weeks old and a mean weight of 29 g (24 g – 34 g) for males and 24 g (18 g – 31 g) for females.
Groups	50/sex/group + 10/sex/group (satellite groups)
Dose	0, 500, 1500 ppm (equal to 85 – 279 mg/kg bw/day in males and 103 – 357 mg/kg bw/day in females)
Route	Oral, dietary
Vehicle	Wessalon (highly dispersed silicates), was added to the powdered food, to improve homogeneity and stability at a ratio of 1:1 (test compound: wessalon)
GLP	Yes
Guideline	OECD-Guideline 453 (1981) Note that the current guideline was adopted in 2009.
Deviation	The following deviations from the current OECD-Guideline 453 (2009) occurred: - Only two dose levels were used due to the study being a follow-up study with the objective to recognise a possible oncogenic potential in the range of elevated dosages - Haematological examination time points only after 12 and 21 months (not at 3 and 6 months) - Urinalyses was not conducted - Weight of the following organs was not determined: epididymides, ovaries, spleen, thyroid (incl. parathyroids) and uterus - The following tissues were not subject to histopathological examination: coagulating gland, lacrimal gland, peripheral nerve
Impact of deviations	Minor – the deviations are minimal, can be compensated by the results of other studies and thus they are not considered to affect the validity of the study
Acceptable	Acceptable
NOAEL	Carcinogenicity: 500 ppm, equivalent to 85 and 103 for males and females respectively. Systemic toxicity: None, as effects were seen from the lowest dose tested.
Effects at the LOAEL	Carcinogenicity: Increased liver tumours in both sexes at 1500 ppm (the top dose). Systemic toxicity: Liver toxicity and changes in some clinical-chemistry and haematological parameters at 500 ppm (equivalent to 85 and 103 for males and females respectively) and above.

Methods

The objective of the study was to investigate further a possible oncogenic effect of tebuconazole in the mouse at higher dose levels than those used in the previous study (B.6.5.2/01). A prior study involving a dose range from 20 – 180 ppm had shown no evidence for an oncogenic potential, but had shown effects on the liver at doses of 60 ppm and above. In addition, slight haematological and clinical chemistry changes had been seen at the top dose of 180 ppm, posing the question as to whether a maximum tolerated dose (MTD) had been reached.

Groups of 50 male and 50 female NMRI mice were administered tebuconazole at concentrations of 0, 500 or 1500 ppm (equal to 0, 85 and 279 mg/kg bw/day in males and 0, 103 and 357 mg/kg bw/day in females) in their diet over a period of 21 months. Groups of 10 male and 10 female animals (satellite groups) were analogously treated, and sacrificed after a study duration of 12 months. To establish the potential presence of chronic toxic effects satellite animals were autopsied after one year and additional haematological and clinical examinations were carried out.

Table 6.5-31. Study design and doses received

Test group		**1**	**2**	**3**
Concentration in diet	[ppm]	**0**	**500**	**1500**
Dose per animal [mg/kg bw/day]	Male	0	85	279
	Female	0	103	357

Results

Clinical observations

The appearance, general behaviour, water intakes and mortality were unaffected at 500 ppm (Table 6.5-31). However, the incidence of animals exhibiting increases in abdominal girth was elevated at the 1500 ppm level. Therefore, clinical signs of toxicity were seen at the top dose of 1500 ppm.

Body weight and food intake

There were no significant negative effects on body weights at week 91 up to the top dose. Food consumption was slightly increased at the highest dose and a dose-response was evident (Table 6.5-31). Overall, no clear adverse effects were seen on body weights and food intake up to the top dose.

Table 6.5-32. Body weight results

	Body Weight – Mean (g)							
	Weeks							
Dose [ppm]	**0**	**4**	**8**	**13**	**25**	**53**	**79**	**91**
	Males							
0	34.0	38.3	39.3	40.5	43.0	46.8	45.7	46.5
500	34.3	38.4	38.4	39.2*	40.5**	45.2	44.1	44.4
1500	34.8**	37.8	37.4**	38.4**	39.0**	42.5**	45.1	46.6
	Weeks							
	0	**4**	**8**	**13**	**25**	**53**	**79**	**91**
	Females							
0	28.1	31.5	31.3	33.2	34.9	38.7	39.6	41.3
500	28.9**	31.3	31.0	33.0	34.3	38.5	39.8	39.3
1500	28.9**	31.6	30.7	32.7	32.6**	36.7*	40.5*	44.1*

* p<0.05; **p<0.01

Table 6.5-33. Results of the combined chronic toxicity/carcinogenicity study in mice (main groups)

	Males			**Females**		
Dose [ppm]	**0**	**500**	**1500**	**0**	**500**	**1500**
Number of animals examined	50	50	50	50	50	50
Mortality (no. animals affected / total no. animals)	20/50	18/50	23/50	30/50	32/50	32/50
Body weight – week 91 [g]	46.5	44.5	46.6	41.3	39.3	**44.1***
Food consumption [g/kg bw/day]	146.6	169.8	186.0	188.8	206.1	237.7
Water consumption [g/kg bw/day]	208.3	213.2	204.1	314.0	297.7	279.4

* significantly different from control $p \leq 0.05$

Haematology and clinical chemistry

The haematological examination did not provide evidence of a clear treatment-related effect in females at 500 ppm. However, marginal reductions in the haematocrit value, and increased MCH and MCHC were present in males at 500 ppm and above. In addition, at 1500 ppm the erythrocyte count, haemoglobin content, haematocrit value and thromboplastin time were generally reduced, whereas the thrombocyte and leukocyte counts were increased, in some cases to a marked extent (Table 6.5-32). Overall, treatment-related effects on some haematological parameters were seen from 500 ppm.

The clinical laboratory tests, gross pathology, organ gravimetry and histopathology provided evidence for marked and dose-related liver damage in both treatment groups. The main clinical-chemistry findings included a marked (statistically significant) increase in the activities of the alanine and aspartate aminotransferases (ALAT and ASAT) and alkaline phosphatase (Table 6.5-32.) from 500 ppm in both sexes. Changes in cholesterol and bilirubin levels were more difficult to interpret as they were inconsistent between time points, and not dose-related. Overall, marked effects on clinical-chemistry parameters indicative of liver damage were seen in both sexes from 500 ppm.

Table 6.5-34. Clinical chemistry and haematology measured in mice treated with tebuconazole in their diet through 21 months

Dose [ppm]		Males 0	Males 500	Males 1500	Females 0	Females 500	Females 1500
ALAT [U/L]	wk 51	38.0	53.2* (40.0%)	236.3** (522 %)	31.7	51.7* (63.1%)	272.5** (760%)
	wk 90	74.9	123.1** (64.4%)	480.8** (541%)	39.2	64.9* (65.6%)	419.4** (970%)
ASAT [U/L]	wk 51	31.9	37.5 (17.6%)	121.3** (280%)	38.3	47.2* (23.2%)	144.0** (276%)
	wk 90	46.1	60.7 (31.7%)	251.8** (446%)	36.9	59.0** (59.9%)	303.8** (723%)
Alkaline phosphatase [U/L]	wk 51	74	117** (58.1%)	181** (145%)	174	212 (21.8%)	292 (67.8%)
	wk 90	126	156 (23.8%)	531** (321%)	182	328 (80.2%)	517** (184%)
Cholesterol [mmol/L]	wk 51	3.71	1.99** (-46.4%)	1.66** (-55.3%)	2.84	1.48** (-47.9%)	1.92*
	wk 90	3.88	1.57** (-59.5%)	4.55 (17.3%)	3.76	2.25** (-40.2%)	3.59
Bilirubin [µmol/L]	wk 51	1.8	1.3** (-27.8%)	1.3** (-27.8%)	2.2	2.1 (-4.5%)	1.9 (-13.6%)
	wk 90	2.1	1.6** (-23.8%)	5.0 (138%)	2.7	2.1*(-22.2%)	4.9 (81.5%)
Inorg. phosphate [mmol/L]	wk 51	1.90	1.73*	2.14*	1.63	1.69 (3.7%)	1.94** (19%)
	wk 90	1.60	1.75**	2.06**	1.62	1.64 (1.2%)	1.93** (19.1%)
Leucocyte count [10^9/L]	wk 51	5.2	5.6 (7.7%)	10.2** (96.2%)	3.9	3.7 (-5.1%)	10.7** (174%)
	wk 90/91	6.6	5.0* (-24.2%)	9.8* (48.5%)	7.6	4.3 (-43.4%)	9.5 (25.0%)
Erythrocyte [10^{12}/L]	wk 51	8.90	9.11	8.51	8.40	8.99 (7.0%)	7.49 (-10.8%)
	wk 90/91	9.13	8.25	7.95** (-12.9%)	8.36	8.63 (3.2%)	7.51(-10.2%)
Haemoglobin [g/L]	wk 51	142	144	129** (-9.2%)	139	148**	132
	wk 90/91	143	150	117** (-18.2%)	132	131	125
Haematocrit [L/L]	wk 51	0.435	0.414	0.373** (-14.3%)	0.422	0.432 (2.4%)	0.381* (-9.7%)
	wk 90/91	0.427	0.375** (-12.2%)	0.375** (-12.2%)	0.407	0.401 (-1.5%)	0.380 (-6.6%)
MCHC [g/L erythrocytes]	wk 51	327	348** (6.4%)	347** (6.1%)	331	344 (3.9%)	347* (4.8%)
	wk 90/91	334	402** (20.4%)	313** (-6.3%)	324	328 (1.2%)	328 (1.2%)
MCH [pg]	wk 51	16.0	15.8	15.3 (-4,4%)	16.6	16.6	17.7 (6.6%)
	wk 90/91	15.7	18.3** (16.6%)	14.6* (-7.0%)	15.8	15.3	16.7 (5.7%)
Thrombocyte count [10^9/L]	wk 51	1311	1323	1650** (25.9%)	1024	1274* (24.4)	1284 (25.4%)
	wk 90/91	1678	1668	2223* (32.5%)	904	1374* (52.0%)	1771** (95.9%)
Clotting time (Hepatoquick) [sec]	wk 51	21.1	20.4	20.1* (-4.7%)	20.7	19.7 (-4.8%)	19.2 (-7.2%)
	wk 90/91	19.0	18.9	16.8** (-11.6%)	19.3	18.8 (-2.6%)	16.3** (-15.5%)

* significantly different from control $p \leq 0.05$
** significantly different from control $p \leq 0.01$
wk week
ASAT: Aspartate aminotransferase ALAT: Alanine aminotransferase
MCHC: Mean corpuscular haemoglobin concentration MCH: Mean corpuscular haemoglobin

Sacrifice and pathology

Non-neoplastic findings

In treated animals from both dose groups, major enlargement of the liver, single cell and focal necrosis, inflammation, bile duct hyperplasia and steatosis were seen (Tables 6.5-33, 6.5-34 and 6.5-37). Liver weights (both relative and absolute) were increased in treated animals, at 52 and 91 weeks; this increase was statistically significant in all treated males and in females at the top dose of 1500 ppm (Tables 6.5-35 and 6.5-36). Adrenals weights were statistically significantly increased in the highest dose (1500 ppm) in females only (Table 6.5-36) at 91 weeks. Overall liver toxicity (increased weight and histopathology) was observed from 500 ppm.

In addition, the histopathology of the interim necropsy animals showed a dose-related increase in the incidence of hyperkeratosis and acanthosis of the forestomach mucosa (Table 6.5-37). These findings were not confirmed at terminal necropsy (Table 6.5-38). On this basis, the effects on the forestomach were not considered treatment-related.

Neoplastic findings

The rate of hepatocellular tumours was unaffected at the 500 ppm level (Table 6.5 42). In contrast, the rates of hepatocellular tumours in males and females were elevated to a highly statistically significant extent at 1500 ppm, and were markedly above the range of spontaneous incidences observed in this mouse strain (Table 6.5-42). Adenomas were increased in males only (35 % at 1500 ppm vs. 6 % in controls), but the carcinomas were increased in both males (21 % at 1500 ppm vs. 0 % in controls) and females (26 % at 1500 ppm vs. 2 % in controls).

Table 6.5-35. Macropathology findings (interim phase)

Macropathology findings - Interim phase						
Dose [ppm]	**0**	**500**	**1500**	**0**	**500**	**1500**
	Males			**Females**		
Liver						
Examined [N]	10	10	10	10	10	10
Accentuated lobular pattern [N]	0	2	1	0	3	4
Appears large [N]	0	0	**10*** **	0	0	**5***
Areas(s) of change [N]	0	1	3	0	0	**6***
Swollen [N]	0	0	0	0	0	2
Irregular surface [N]	0	0	0	0	0	1
Pale [N]	0	**5***	**9*** **	0	**6***	**8*** **

* significantly different from control $p \leq 0.05$

*** significantly different from control $p \leq 0.001$

Statistically significant values are written in **bold letters**.

Table 6.5-36. Macropathology findings (terminal phase)

Macropathology findings - Terminal phase						
Dose [ppm]	**0**	**500**	**1500**	**0**	**500**	**1500**
	Males			**Females**		
Liver						
Examined [N]	50	50	50	50	50	50
Accentuated lobular pattern [N]	1	7	1	0	5	1
Appears large [N]	1	2	**35*** **	0	5	**32*** **
Areas(s) of change [N]	0	1	2	0	0	3
Swollen [N]	0	1	0	1	1	3
Irregular surface [N]	1	0	**30*** **	3	3	**26*** **
Mass(es) [N]	6	3	**13** **	1	1	**8***
Cystic [N]	0	0	1	0	0	0

Macropathology findings - Terminal phase						
Dose [ppm]	**0**	**500**	**1500**	**0**	**500**	**1500**
Firm [N]	0	0	1	0	0	0
Adhesion(s) [N]	0	0	0	0	0	1
Pale [N]	2	0	2	0	5	5

* significantly different from control $p \leq 0.05$
** significantly different from control $p \leq 0.01$
*** significantly different from control $p \leq 0.001$
Statistically significant values are written in **bold letters**.

Table 6.5-37. Organ weight results (interim phase, 52 weeks)

Organ weights, absolute [mg]							
Dose [ppm]	**Body weight [g]**	**Brain**	**Heart**	**Testes**	**Liver**	**Kidneys**	**Adrenals**
Males							
0	43	533	280	271	1963	810	9
500	48	514	267	287	**2737** ** **(39.4%)**	763	12
1500	43	508	252	262	**4159** ** **(112%)**	763	10
Females							
0	38	534	258	-	2131	629	16
500	38	541	**209** **	-	2426 (13.8%)	**499** ** **(-20.7%)**	15
1500	38	532	**213** **	-	**4328** ** **(103%)**	**512** ** **(-17.6%)**	20
Organ weights, relative [mg/100 g]							
Males							
0	43	1233	653	618	4529	1880	21
500	48	1074	556	597	**5666** ** **(25.1%)**	1592 (-15.3%)	26
1500	43	1181	583	611	**9606** ** **(112.1%)**	1770 (-5.9%)	24
Females							
0	38	1401	670	-	5520	1643	42
500	38	1452	**558** **	-	**6463** * **(17.1%)**	**1340** ** **(-18.4%)**	39
1500	38	1416	**568** **	-	**11392** ** **(106.4%)**	**1368** * **(-16.7%)**	53

* significantly different from control $p \leq 0.05$
** significantly different from control $p \leq 0.01$
Statistically significant values are written in **bold letters**.

Table 6.5-38. Organ weight results (terminal kill, 91 weeks)

Organ weights, absolute [mg]							
Dose [ppm]	**Body weight [g]**	**Brain**	**Heart**	**Testes**	**Liver**	**Kidneys**	**Adrenals**
Males							

0	46	512	285	247	2409	906	11
500	44	500	281	227	**2822** ** **(17.1%)**	**797** * **(-12%)**	10
1500	47	**487** **	307	228	**8522** ** **(254%)**	**771** ** **(-14.9%)**	11
Females							
0	41	526	249	-	2524	541	12
500	39	519	247	-	2623 (3.9%)	524	13
1500	**44** *	506	261	-	**9405** ** **(277%)**	594	**15** ** **(25%)**
Organ weights, relative [mg/100 g]							
Males							
0	46	1113	617	534	5214	1947	24
500	44	1133	635	514	**6345** ** **(21.7%)**	1800 (-7.6%)	21
1500	47	1055	665	492	**18313** ** **(251%)**	**1668** ** **(-14.3%)**	23
Females							
0	41	1282	609	-	6060	1317	28
500	39	1327	634	-	6642 (9.6%)	1342	33
1500	**44** *	**1155** **	592	-	**21141** ** **(249%)**	1348	**34** * **(21%)**

* significantly different from control $p \leq 0.05$
** significantly different from control $p \leq 0.01$
Statistically significant values are written in **bold letters**.

Table 6.5-39. Non-neoplastic findings – Interim phase

Organs		**Dose [ppm]**					
		0	**500**	**1500**	**0**	**500**	**1500**
		Males			**Females**		
Liver							
Examined	[N]	10	9	10	10	10	10
Focal inflammation with associated hepatocytic generation	[N]	1	5	1	0	**5***	2
Necrosis of individual hepatocytes	[N]	0	**5***	**8*****	0	2	**9*****
Focal necrosis	[N]	0	0	0	0	1	2
Focal hyperplasia of hepatocytes	[N]	0	0	2	0	0	3
Periacinar hepatocytic vacuolation	[N]	0	1	0	0	0	0
Panacinar fine fatty vacuolation	[N]	0	**8*****	**10*****	0	**10*****	**10*****
Centriacinar fatty vacuolation (large)	[N]	1	1	1	0	**9*****	**6***
Periacinar hepatocytic hypertrophy	[N]	0	0	0	0	0	1
Chronic inflammatory cells within the portal area	[N]	1	3	2	1	4	**8****
Bile duct hyperplasia	[N]	0	1	**8*****	0	2	**6***
Periportal fibrosis	[N]	0	1	**5***	0	0	2
Extramedullary haemopoiesis	[N]	0	1	1	0	3	**5***
Eosinophilic focus/foci of hepatocellular alteration	[N]	0	0	0	0	0	3

Organs		Dose [ppm]					
		0	**500**	**1500**	**0**	**500**	**1500**
Pigment laden Kupffer cells	[N]	0	**4***	**8*****	0	0	**8*****
Fat stain: Panacinar fat	[N]	1	**6***	**8****	6	9	5
Fat stain: Pericinar fat	[N]	2	3	2	0	0	**5***
Focal mineralization	[N]	0	0	1	0	0	0
Stomach							
Examined	[N]	10	9	10	10	10	10
Glandular region:							
Acute inflammation	[N]	1	0	0	0	0	0
Chronic inflammation	[N]	0	1	0	0	1	0
Dysplasia	[N]	1	0	0	0	0	0
Keratinized region:							
Hyperkeratosis and acanthosis	[N]	1	2	6	2	6	**8***

* significantly different from control $p \leq 0.05$
** significantly different from control $p \leq 0.01$
*** significantly different from control $p \leq 0.001$
Statistically significant values are written in **bold letters**.

Table 6.5-40. Non-neoplastic findings – Terminal phase

Organs		Dose [ppm]					
		0	**500**	**1500**	**0**	**500**	**1500**
		Males			**Females**		
Liver							
Examined	[N]	47	48	48	47	45	46
Focal inflammation with associated hepatocytic generation	[N]	1	1	0	3	2	0
Necrosis of individual hepatocytes	[N]	3	**11***	2	0	2	1
Focal necrosis	[N]	1	1	1	1	3	5
Focal hyperplasia of hepatocytes	[N]	6	2	**23*****	1	0	**12*****
Kupffer cell hyperplasia	[N]	0	3	3	0	0	1
Panacinar hepatocytic fatty vacuolation	[N]	0	5	0	0	0	0
Panacinar fine fatty vacuolation	[N]	0	**14*****	**25*****	1	4	**19*****
Centriacinar fatty vacuolation (large)	[N]	1	1	0	3	**13****	4
Periacinar hepatocytic hypertrophy	[N]	0	0	2	0	0	**13*****
Chronic inflammatory cells within the portal area	[N]	2	2	7	5	1	2
Bile duct hyperplasia	[N]	0	3	5	0	0	1
Oval cells proliferation	[N]	0	0	**23*****	0	0	**17*****
Extramedullary haemopoiesis	[N]	0	2	**7***	5	1	12
Clear cell focus/foci	[N]	0	0	2	0	0	4
Eosinophilic focus/foci of hepatocellular alteration	[N]	0	2	3	0	0	**7****
Biliary cyst(s)	[N]	0	0	2	0	0	0
Pigment-laden Kupffer cells	[N]	1	0	6	1	3	**7***
Focal telangiectasis	[N]	0	0	1	0	0	0
Amyloidosis	[N]	1	1	0	1	0	0

Organs		Dose [ppm]					
		0	**500**	**1500**	**0**	**500**	**1500**
Focal mineralization	[N]	0	0	0	0	0	1
Vascular damage and associated focal epithelial hyperplasia	[N]	0	0	2	0	0	0
Stomach							
Examined	[N]	47	48	48	46	45	46
Glandular region:							
Acute inflammation	[N]	0	0	0	1	0	0
Chronic inflammation	[N]	0	0	0	1	0	0
Dysplasia	[N]	16	**7***	**2*****	14	7	11
Keratinized region:							
Hyperkeratosis and acanthosis	[N]	6	8	8	12	16	13

* significantly different from control $p \leq 0.05$
** significantly different from control $p \leq 0.01$
*** significantly different from control $p \leq 0.001$
Statistically significant values are written in **bold letters**.

Table 6.5-41. Animals with tumours – Interim phase

Tumours		Dose [ppm]					
		0	**500**	**1500**	**0**	**500**	**1500**
		Males			**Females**		
Primary Tumour	[N]	0	1	0	3	0	1
Benign Tumour	[N]	0	1	0	0	0	0
Malignant Tumour	[N]	0	0	0	3	0	1

Table 6.5-42. Animals with neoplastic findings – Interim phase

Neoplastic findings		Dose [ppm]					
		0	**500**	**1500**	**0**	**500**	**1500**
		Males			**Females**		
Caecum							
Examined	[N]	10	9	10	10	10	10
Leiomyoma (b)	[N]	0	1	0	0	0	0
Hematopoietic System							
Examined	[N]	10	10	10	10	10	10
Lymphoma (m)	[N]	0	0	0	3	0	1

(b) = benign neoplasms; (m) = malignant neoplasms

Table 6.5-43. Animals with tumours – Terminal phase

Tumours		Dose [ppm]					
		0	**500**	**1500**	**0**	**500**	**1500**
		Males			**Females**		
Primary Tumour	[N]	21	23	34	31	30	30
Benign Tumour	[N]	15	15	23	16	12	6
Malignant Tumour	[N]	9	10	20	22	22	27

Table 6.5-44. Animals with neoplastic findings – Terminal phase

Neoplastic findings	Dose [ppm]					
	0	**500**	**1500**	**0**	**500**	**1500**
	Males			**Females**		
Adrenals						
Examined [N]	47	48	48	47	44	46
Pheochromocytoma (b) [N]	0	1	2	0	0	0
Cortical Adenoma (b) [N]	2	0	0	0	0	0
Pheochromocytoma (m) [N]	1	0	0	0	0	0
Harderian Gland						
Examined [N]	50	50	49	50	49	50
Adenoma (b) [N]	4	6	2	2	1	1
Kidneys						
Examined [N]	47	48	48	47	45	46
Carcinoma (m) [N]	0	0	0	0	1	0
Liver						
Examined	47	48	48	47	45	46
Hepatocellular Adenoma (b) [N]	3 (6%)	2 (4%)	**17*** (35%)**	0 (0%)	0 (0%)	2 (4%)
Hepatocellular Carcinoma (m) [N]	0 (0%)	0 (0%)	**10*** (21%)**	1 (2%)	0 (0%)	**12*** (26%)**
Hemangiosarcoma [N]	0	0	0	0	1	0
Lungs						
Examined [N]	47	48	48	47	44	46
Pulmonary Adenoma (b) [N]	8	6	10	3	4	3
Pulmonary Carcinoma (m) [N]	1	1	2	1	0	0
Mammary Area						
Examined [N]	47	48	47	47	45	47
Adenocarcinoma (m) [N]	0	0	0	1	2	1
Adenoacanthoma (m) [N]	0	0	0	1	0	0
Oesophagus						
Examined [N]	49	49	49	49	45	46
Squamous Cell Papilloma (b) [N]	0	0	1	0	0	0
Ovaries						
Examined [N]	-	-	-	47	44	45
Haemangioma (b) [N]	-	-	-	1	0	0
Granulosa Cell Tumour (b) [N]	-	-	-	1	5	0
Luteal Cell Tumour (b) [N]	-	-	-	1	1	1
Hemangiosarcoma (m) [N]	-	-	-	1	0	0
Pancreas						
Examined [N]	47	48	48	45	44	46
Islet Cell Adenoma (b) [N]	0	0	0	0	1	0
Pituitary						
Examined [N]	49	48	49	49	47	46
Adenoma (b) [N]	0	0	0	7	1	0
Testes						
Examined [N]	47	48	48	-	-	-

Neoplastic findings	Dose [ppm]					
	0	**500**	**1500**	**0**	**500**	**1500**
Interstitial Cell Tumour (b) [N]	0	1	0	-	-	-
Uterus						
Examined [N]	-	-	-	47	45	45
Leiomyoma (b) [N]	-	-	-	1	0	0
Haematopoietic Tissue						
Examined [N]	48	49	48	47	45	46
Lymphoma (m) [N]	6	7	7	21	16	14
Histiocytic Sarcoma (m) [N]	1	2	3	1	3	5
Perianal Glands						
Examined [N]	2	4	5	-	-	-
Cystadenoma [N]	0	0	1	-	-	-
Tail						
Examined [N]	2	0	0	0	0	0
Fibroma (b) [N]	1	0	0	0	0	0

* significantly different from control $p \leq 0.05$
** significantly different from control $p \leq 0.01$
*** significantly different from control $p \leq 0.001$
Statistically significant values are written in **bold letters**.
(b) = benign neoplasms; (m) = malignant neoplasms

Conclusion
In conclusion, administration of tebuconazole to mice for 21 months caused severe liver effects at doses of 500 ppm (equivalent to 85 and 103 mg/kg bw/day for males and females respectively) and above. Effects included enlargement of the liver, increased liver weights, single cell and focal necrosis, inflammation, bile duct hyperplasia and steatosis. These were accompanied by clinical-chemistry and haematological changes. Given the marked liver toxicity observed in particular at 1500 ppm, it is considered that the MTD was exceeded at the top dose. Liver tumours were significantly increased in both sexes at 1500 ppm (279 and 357 mg/kg bw/day in males and females), a dose at which marked liver toxicity occurred.

A NOAEL for carcinogenicity was determined at 500 ppm (equivalent to 85 and 103 mg/kg bw/day for males and females, respectively) based upon an increased incidence of liver tumours at the top dose of 1500 ppm (279 and 357 mg/kg bw/day in males and females).

A systemic LOAEL of 500 ppm (equivalent to 85 and 103 mg/kg bw/day for males and females, respectively) was identified in this study, based on liver toxicity and effects on some clinical-chemistry and haematological parameters.

B.6.5.3. Additional mechanistic information on the mouse liver tumours

A number of *in vivo* and *in vitro* mechanistic studies have been performed to elucidate the most likely mode of action underpinning the formation of liver tumours observed in male and female NMRI mice at the top dose of 1500 ppm (279/353 mg/kg bw/d in M/F). These are all new studies submitted by the Bayer Task Force for the purpose of renewal.

Previous evaluation	None – submitted for the purpose of renewal (study owned by Bayer Task force)

Study ID	B.6.5.3/01
Study title	Tebuconazole - 28-day liver mechanistic study in the male and female mice by dietary administration (liver enzyme activity and gene transcript investigation)
Test substance	Tebuconazole
Purity (%)	97.5

Batch no.	K689052
GLP	No
Guideline	n/a
Deviation	n/a
Acceptable	Acceptable as a mechanistic study

Methods
Tebuconazole was administered continuously via the diet to groups of NMRI mice (20/sex/group) for at least 7 or 28 days at concentrations of 0, 25, 500 and 1500 ppm (equating approximately to 4, 72 and 201 mg/kg bw/day in males and 5, 87 and 273 mg/kg bw/day in females for the 7-day exposure period and equating approximately to 4, 77 and 231 mg/kg bw/day in males and 5, 90 and 276 mg/kg bw/day in females for the 28-day exposure period) to investigate liver enzyme induction (enzyme activity and gene transcripts). An additional group received Phenobarbital (suspension in 0.5 % aqueous solution of methylcellulose) by oral gavage once per day at 80 mg/kg bw/day and acted as positive control for enzyme induction. Clinical observations, body weights and food consumption were investigated throughout the duration of the study. Blood samples were taken from the 28-day exposed animals on day 30 for investigation of clinical-chemistry parameters. At sacrifice, liver weights were measured in the 28-day exposed animals while liver macroscopic and microscopic investigations were performed in both the 7-day and 28-day exposed animals. Liver samples were taken at sacrifice from both the 7-day and 28-day exposed animals to analyse gene transcripts by quantitative PCR of a number of enzymes and proteins. In addition, total and specific cytochrome P-450 enzyme activities were measured in liver samples from the 28-day exposed animals.

Results

7-day exposure:
There was a reduction of mean body weight due to a mean body weight loss and a cumulative body weight loss at 1500 ppm in both sexes. In addition, at necropsy, at 1500 ppm, mean terminal body weight was statistically significantly lower (-8 %), $p \leq 0.01$) in both sexes when compared to controls.

At 1500 ppm, mean food consumption was reduced by 19 % ($p \leq 0.01$) in males only compared to controls.

At 1500 ppm, enlarged liver was noted (in all animals) as well as prominent lobulation of liver (4/20 males), pale liver (3/20 males and 12/20 females), and white foci on liver (8/20 males). At 500 ppm, enlarged liver was noted (18/20 males and females) as well as pale liver (5/20 males and 14/20 females) and white foci on liver in 3/20 males.

Table 6.5-45. Incidence of macroscopic changes in the liver (n = 20)

	Tebuconazole [ppm]				**PB [mg/kg bw]**	**Tebuconazole [ppm]**				**PB [mg/kg bw]**
	0	**25**	**500**	**1500**	**80**	**0**	**25**	**500**	**1500**	**80**
	Males					**Females**				
7-day exposure										
Enlarged	0	1	18	20	--	0	2	18	20	--
Pale	0	0	5	3	--	0	0	14	12	--
Focus(i), white	0	0	3	8	--	1	1	0	0	--
Prominent lobulation	0	0	0	4	--	0	0	0	1	--
28-day exposure										
Enlarged	0[#]	0	14	19	10	0	0	12	20	15
Pale	1[#]	0	3	2		2	4	11	11	
Dark	2[#]	0	0	0	14	0	0	0	0	9
Focus(i), white	0	0	0	0	0	0	0	0	0	8
Prominent lobulation	5[#]	5	4	9		0	1	1	0	

[#]: only 19 animals were assessed
PB: Phenobarbital
--: not tested

At 1500 ppm in the liver, the most highly up-regulated phase I enzyme gene transcripts, both in males and females, were Cyp 2B10 and Cyp 3A11 when compared to the controls. Cyp 1A1 and Cyp 2B9 gene transcripts were slightly deregulated (up and down-regulated, respectively) in the female mice only. Regarding the phase II enzyme, UgtlAl gene transcripts were slightly up and down-regulated in males and females, respectively whereas Ugt2Bl gene transcripts were slightly down-regulated in male mice only when compared to the controls. The pro-apoptotic gene transcripts Bax were slightly up-regulated (in both sexes) whereas the anti-apoptotic gene transcripts Bcl-Xl (males only) were slightly down-regulated. Growth arrest and DNA-damage inducible protein GADD45 alpha (Gadd45a, increased following stressful growth arrest conditions) gene transcripts were up-regulated in the female mice. The peroxisomal Acox1 gene transcripts were slightly down-regulated in the males only.

At 500 ppm in the liver, the most highly upregulated phase I gene transcripts, both in males and females, were Cyp 2B10 and Cyp 3A11. The unique phase II enzyme gene transcripts deregulated were Ugt1A1, slightly up-regulated in the male mice only. The pro-apoptotic gene transcripts Bax were still slightly up-regulated in both sexes.

In males only, at 25 ppm only Cyp 2B10 and Cyp 3A11 gene transcripts in the liver were up-regulated.

Table 6.5-46. Mean Relative Quantity ± standard deviation of gene transcripts (% change compared to control mean values) after 7-day and 28-day exposure

		Tebuconazole [ppm]				**PB [mg/kg bw]**
		0	**25**	**500**	**1500**	**80**
Males						
Cyp 1a1	7	1.24 ± 0.29	1.74 ± 0.39 *(+41 %)*	2.26 ± 0.79 *(+82 %)*	2.18 ± 0.40 *(+76 %)*	--
	28	1.16 ± 0.44	1.40 ± 0.41 *(+21 %)*	1.67 ± 0.63 *(+43 %)*	1.51 ± 0.40 *(+30 %)*	1.28 ± 0.25 *(+10 %)*
Cyp 2b9	7	3.50 ± 3.37	40.24 ± 48.01 *(+1051 %)*	6.22 ± 3.47 *(+78 %)*	65.20 ± 71.72 *(+1766 %)*	--
	28	0.39 ± 0.42	0.28 ± 0.16 *(-29 %)*	0.31 ± 0.21 *(-20 %)*	**12.42 ± 4.85** ***(+1203 %)***	**2.86 ± 0.68** ***(+636 %)***
Cyp2b10	7	1.21 ± 0.34	**4.33 ± 1.84** ***(+258 %)***	**30.03 ± 8.38** ***(+2384 %)***	**34.78 ± 7.37** ***(+2760 %)***	--
	28	1.28 ± 0.49	**9.46 ± 6.29** ***(+640 %)[a]***	**65.82 ± 18.11** ***(+6454 %)***	**73.38 ± 13.26** ***(+5637 %)***	**148.01 ± 12.34** ***(+11472 %)***
Cyp3a11	7	1.07 ± 0.12	**1.76 ± 0.13** ***(+64 %)[a]***	**7.34 ± 0.84** ***(+586 %)***	**9.11 ± 1.27** ***(+751 %)***	--
	28	0.91 ± 0.17	1.25 ± 0.20 *(+38 %)*	**5.38 ± 1.31** ***(+491 %)***	**6.80 ± 0.80** ***(+647%)***	**4.52 ± 0.46** ***(+397 %)***
Cyp4a10	7	0.98 ± 0.08	0.96 ± 0.17 *(-2 %)*	0.74 + 0.18 *(-24 %)*	**0.37 ± 0.08** ***(-62 %)***	--
	28	1.05 ± 0.29	0.87 ± 0.14 *(-17 %)*	**0.56 ± 0.10** ***(-46 %)***	**0.36 ± 0.01** ***(-66 %)***	**0.59 ± 0.09** ***(-44 %)***
Ugt1a1	7	1.29 ± 0.30	1.23 ± 0.17 *(-5 %)*	**1.99 ± 0.45** ***(+53 %)***	**2.28 ± 0.25** ***(+76 %)***	--
	28	0.89 ± 0.15	0.87 ± 0.03 *(-3 %)*	0.99 ± 0.16 *(+12 %)*	**1.28 ± 0.11** ***(+44 %)***	**2.33 ± 0.22** ***(+162 %)***
Ugt2b1	7	1.33 ± 0.26	1.24 ± 0.20 *(-7 %)*	1.41 ± 0.13 *(+6 %)*	**0.88 ± 0.05** ***(-34 %)***	--
	28	0.93 ± 0.31	0.85 ± 0.06 *(-9 %)*	0.78 ± 0.08 *(-16 %)*	**0.57 ± 0.10** ***(-39 %)***	1.08 ± 0.16 *(+16 %)*
Acox1	7	1.04 ± 0.17	0.94 ± 0.08 *(-10 %)*	0.99 ± 0.26 *(-5 %)*	**0.68 ± 0.07** ***(-35 %)***	--
	28	0.94 ± 0.19	0.94 ± 0.07 *(±0 %)*	0.89 ± 0.16 *(-6 %)*	**0.66 ± 0.05** ***(-30 %)***	**0.54 ± 0.03** ***(-42%)***
Bax	7	0.99 ± 0.03	1.03 ± 0.11 *(+4 %)*	**1.36 ± 0.14** ***(+38 %)***	**1.43 ± 0.13** ***(+45 %)***	--

		Tebuconazole [ppm]				PB [mg/kg bw]
		0	**25**	**500**	**1500**	**80**
	28	1.08 ± 0.11	1.23 ± 0.12 *(+14 %)*	1.38 ± 0.21 *(+28 %)*	1.34 ± 0.11 *(+23 %)*	1.08 ± 0.05 *(±0 %)*
Bcl-X1	7	0.98 ± 0.06	0.91 ± 0.11 *(-7 %)*	0.83 ± 0.03 *(-15 %)*	**0.75 ± 0.11** ***(-34%)***	--
	28	1.34 ± 0.27	1.27 ± 0.21 *(-5 %)*	1.23 ± 0.08 *(-8 %)*	1.14 ± 0.16 *(-15 %)*	1.65 ± 0.38 *(+23 %)*
Ccnb1	7	0.90 ± 0.14	17.21 ± 23.73 *(+1810 %)*	4.08 ± 1.05 *(+353 %)*	59.06 ± 18.66 *(+6455 %)*	--
	28	18.17 ± 30.04	0.71 ± 0.08 *(-96 %)*	0.75 # *(-96 %)*	15.06 ± 8.22 *(-17 %)*	2.23 ± 0.55 *(-88 %)*
Gadd45a	7	1.12 ± 0.16	1.46 ± 0.75 *(+31 %)*	3.63 ± 5.03 *(+225 %)*	1.63 ± 0.72 *(+46 %)*	--
	28	1.88 ± 0.86	1.15 ± 0.29 *(-39 %)*	0.87 ± 0.27 *(-54 %)*	0.98 ± 0.21 *(-48 %)*	2.66 ± 2.60 *(-41 %)*
Females						
Cyp 1a1	7	1.04 ± 0.26	1.14 ± 0.42 *(+ 9)*	1.01 ± 0.17 *(-4 %)*	*1.80 ± 0.25* *(+73 %)*	--
	28	1.44 ± 0.36	2.26 ± 1.25 *(+57 %)*	1.68 ± 0.35 *(+17 %)*	2.13 ± 0.53 *(+48 %)*	1.78 ± 0.55 *(+24 %)*
Cyp 2b9	7	1.00 ± 0.04	1.30 ± 0.21 *(+30 %)*	1.01 ± 0.18 *(+1 %)*	**0.62 ± 0.17** ***(-38 %)***	--
	28	1.14 ± 0.16	1.16 ± 0.12 *(+2 %)*	**0.68 ± 0.19** ***(-40 %)***	**0.46 ± 0.08** ***(-59 %)***	**0.52 ± 0.10** ***(-55 %)***
Cyp2b10	7	0.65 ± 0.26	0.95 ± 0.64 *(+46 %)*	**4.70 ± 1.68** ***(+624 %)***	**5.28 ± 0.46** ***(+712 %)***	--
	28	1.17 ± 0.52	3.76 ± 1.82 *(+221 %)*	**11.71 ± 2.97** ***(+897 %)***	**13.74 ± 1.71** ***(+1070 %)***	**38.22 ± 8.17** ***(+3155 %)***
Cyp3a11	7	0.70 ± 0.30	1.03 ± 0.31 *(+49 %)*	**3.63 ± 0.69** ***(+422 %)***	**6.69 ± 0.60** ***(+863 %)***	--
	28	1.00 ± 0.22	0.83 ± 0.19 *(-17 %)*	**2.80 ± 0.64** ***(+180 %)***	**5.23 ± 0.23** ***(+423 %)***	**4.48 ± 0.21** ***(+348 %)***
Cyp4a10	7	0.96 ± 0.06	0.84 ± 0.18 *(-12 %)*	0.83 ± 0.14 *(-13 %)*	**0.40 ± 0.10** ***(-58 %)***	--
	28	0.90 +0.11	0.90 + 0.15 *(+1 %)*	**0.62 ± 0.17** ***(-30 %)***	**0.36 ± 0.09** ***(-60 %)***	**0.36 ± 0.02** ***(-60 %)***
Ugt1a1	7	0.96 ± 0.06	1.07 ± 0.16 *(+12 %)*	0.93 ± 0.04 *(-4 %)*	**0.76 ± 0.06** ***(-20 %)***	--
	28	1.18 ± 0.20	1.17 ± 0.16 *(-1 %)*	0.90 ± 0.14 *(-24 %)*	**0.65 ± 0.08** ***(-45 %)***	**2.14 ± 0.28** ***(+81 %)***
Ugt2b1	7	1.07 ± 0.16	1.17 ± 0.36 *(+9 %)*	0.96 ± 0.10 *(-10 %)*	0.86 ± 0.17 *(-20 %)*	--
	28	1.25 ± 0.28	1.08 ± 0.24 *(-13 %)*	**0.80 ± 0.24** ***(-36 %)***	**0.65 ± 0.06** ***(-48 %)***	1.16 ± 0.19 *(-7 %)*
Acox1	7	1.02 ± 0.10	1.04 ± 0.16 *(+2 %)*	1.17 ± 0.10 *(+15 %)*	0.83 ± 0.13 *(-18 %)*	--
	28	0.97 ± 0.14	0.96 ± 0.09 *(-2 %)*	**0.55 ± 0.08** ***(-43 %)***	**0.52 ± 0.04** ***(-46 %)***	**0.45 ± 0.07** ***(-54 %)***
Bax	7	0.99 ± 0.11	1.21 ± 0.12 *(+23 %)*	**1.65 ± 0.20** ***(+68 %)***	**2.13 ± 0.25** ***(+116 %)***	--
	28	1.09 ± 0.07	1.05 ± 0.14 *(-4 %)*	1.05 ± 0.17 (-4 %)	1.35 ± 0.18 *(+23 %)*	**1.10 ± 0.13** ***(+1 %)***
Bcl-X1	7	0.90 ± 0.13	1.04 ± 0.25 *(+15 %)*	0.94 ± 0.21 *(+4 %)*	0.86 ± 0.05 *(-5 %)*	--
	28	0.97 ± 0.05	0.92 ± 0.27	0.79 ± 0.08	0.75 ± 0.06	**0.80 ± 0.12**

		Tebuconazole [ppm]				PB [mg/kg bw]
		0	**25**	**500**	**1500**	**80**
			(-5 %)	*(-18 %)*	*(-22 %)*	***(-17 %)***
Ccnb1	7	0.75 ± 0.35	0.91 ± 1.30 *(+22 %)*	0.29 ± 0.16 *(-61 %)*	2.42 ± 0.74 *(+224 %)*	--
	28	0.47 ± 0.38	0.34 ± 0.32 *(-27 %)*	0.54 ± 0.30 *(+14 %)*	1.60 ± 1.35 (+238 %)	0.26 ± 0.24 *(-46 %)*
Gadd45a	7	1.50 ± 0.41	2.07 ± 0.50 *(+38 %)*	2.56 ± 1.74 *(+71 %)*	**5.83 ± 2.54** ***(+289 %)***	--
	28	1.51 ± 0.60	0.83 ± 0.22 *(-45 %)*	**0.77 ± 0.36** ***(-49 %)***	**0.61 ± 0.24** ***(-60 %)***	1.82 ± 1.02 *(+21 %)*

Values in bold and bold combined with italics are significantly different from control at $p \leq 0.05$ or $p \leq 0.01$
--: not applicable
#: SD missing

28-day exposure:
At 1500 ppm in both sexes a reduction of mean body weight due to a mean body weight loss and a cumulative body weight loss was observed. At necropsy, at 1500 ppm, a lower mean terminal body weight (-10 %, $p \leq 0.01$) was statistically significant in males when compared to the controls.

In males, at 1500 ppm, mean food consumption was reduced between 6.6 % ($p \leq 0.01$, Study Day 22) and 9.8 % ($p \leq 0.01$, Study Day 15) compared to the controls during the first three weeks. No treatment-related effects were observed at Study Day 29. In females, at 1500 ppm, mean food consumption was reduced by 12.5 % ($p \leq 0.01$) at Study Day 8 only.

Clinical chemistry analyses revealed treatment-related changes (lower total cholesterol, lower total bilirubin in males, slightly lower total protein and albumin and higher aminotransferases and alkaline phosphatase) at 1500 and 500 ppm in both sexes. At 25 ppm, no toxicologically relevant changes were noted.

Table 6.5-47. Clinical chemistry after 28-day exposure (mean ± standard deviation)

	Tebuconazole [ppm]				PB [mg/kg bw]
	0	**25**	**500**	**1500**	**80**
males					
Total cholesterol [mmol/L] *(%)[a]*	3.47 ± 0.53	3.77 ± 0.48 *(+9)*	**1.16 ± 0.41**** ***(-67)***	**0.61 ± 0.36**** ***(-82)***	3.25 ± 0.52 *(-6)*
Total bilirubin [µmol/L] *(%)[a]*	2.1 ± 0.9	2.0 ± 0.4 *(-5)*	**1.2 ± 0.3**** ***(-43)***	**1.1 ± 0.3**** ***(-48)***	**0.9 ± 0.8**** ***(-57)***
Aspartate aminotransferase [IU/L] *(%)[a]*	174 ± 110	96 ± 37 *(-45)*	213 ± 142 *(+22)*	**361 ± 142**** ***(+107)***	166 ± 79 *(-5)*
Alanine aminotransferase [IU/L] *(%)[a]*	61 ± 43	50 ± 49 *(-18)*	105 ± 39 *(+72)*	**417 ± 265**** ***(+584)***	**103 ± 59*** ***(+69)***
Alkaline phosphatase [IU/L] *(%)[a]*	64 ± 18	75 ± 23 *(+17)*	**108 ± 15**** ***(+69)***	**215 ± 51**** ***(+236)***	**109 ± 33**** ***(+70)***
Total protein [g/L] *(%)[a]*	55 ± 5	55 ± 2 *(±0)*	**48 ± 2**** ***(-13)***	**50 ± 2**** ***(-9)***	53 ± 3 *(-4)*
Albumin [g/L] *(%)[a]*	32 ± 2	32 ± 1 *(±0)*	**29 ± 2**** ***(-9)***	**30 ± 1**** ***(-6)***	**31 ± 1*** ***(-3)***
females					
Total cholesterol [mmol/L] *(%)[a]*	2.95 ± 0.49	2.99 ± 0.46 *(+1)*	**0.77 ± 0.33**** ***(-74)***	**0.62 ± 0.34**** ***(-79)***	**2.39 ± 0.64**** ***(-19)***
Total bilirubin [µmol/L] *(%)[a]*	1.6 ± 0.5	1.7 ± 0.4 *(+6)*	1.5 ± 0.7 *(-6)*	1.5 ± 0.6 *(-6)*	**0.4 ± 0.3**** ***(-75)***

	Tebuconazole [ppm]				PB [mg/kg bw]
	0	**25**	**500**	**1500**	**80**
Aspartate aminotransferase [IU/L]	129 ± 39	155 ± 121	**313 ± 187****	**317 ± 90****	**200 ± 51****
(%)[a]		*(+20)*	***(+143)***	***(+146)***	***(+55)***
Alanine aminotransferase [IU/L]	52 ± 33	44 ± 17	**128 ± 60****	**338 ± 127****	**130 ± 73****
(%)[a]		*(-15)*	***(+146)***	***(+550)***	***(+150)***
Alkaline phosphatase [IU/L]	116 ± 23	123 ± 30	**249 ± 250****	**237 ± 61****	117 ± 29
(%)[a]		*(+6)*	***(+115)***	***(+104)***	*(+1)*
Total protein [g/L][a]	57 ± 2	57 ± 2	52 ± 3**	49 ± 3**	53 ± 2**
(%)[a]		*(±0)*	*(-9)*	*(-14)*	*(-7)*
Albumin [g/L]	36 ± 2	36 ± 1	32 ± 1**	31 ± 2**	32 ± 1**
(%)[a]		*(±0)*	*(-11)*	*(-14)*	*(-11)*

[a] % change compared to control
** $p \leq 0.01$
PB: Phenobarbital

At 1500 ppm and at 500 ppm, mean absolute and relative liver weights were statistically significantly higher when compared to controls in both sexes.

Table 6.5-48. Organ weights after 28-day exposure (mean ± standard deviation)

	Tebuconazole [ppm]				PB [mg/kg bw]
	0	**25**	**500**	**1500**	**80**
males					
Absolute liver weight [g]	1.47±0.18	1.41 ± 0.14	**1.98 ± 0.21****	**2.39 ± 0.36****	**1.70±0.25****
(%)[a]		*(-4)*	***(+35)***	***(+63)***	***(+16)***
Liver / body weight ratio [%]	4.22 ±0.37	4.14 ± 0.26	**5.91 ± 0.45****	**7.60 ± 0.87****	**5.19 ± 0.62****
(%)[a]		*(-2)*	***(+40)***	***(+80)***	***(+23)***
Liver / brain weight ratio [%]	325.19 ± 41.40	313.67 ± 35.34	**435.51 ± 45.41****	**535.97 ± 90.91****	**378.37 ± 55.60****
(%)[a]		*(-4)*	***(+34)***	***(+65)***	***(+16)***
females					
Absolute liver weight [g]	1.21 ± 0.10	1.24 ± 0.12	**1.63 ± 0.23****	**1.95 ± 0.29****	**1.58 ± 0.26****
(%)[a]		*(+2)*	***(+35)***	***(+61)***	***(+31)***
Liver / body weight ratio [%]	4.068 ± 0.245	4.322 ± 0.372	**5.758 ± 0.642****	**6.946 ± 0.690****	**5.587 ± 0.619****
(%)[a]		*(+6)*	***(+42)***	***(+71)***	***(+37)***
Liver / brain weight ratio [%]	258.298 ± 20.096	267.159 ± 28.469	**345.651 ± 52.236****	**432.821 ± 60.259****	**349.192 ± 60.390****
(%)[a]		*(+3)*	***(+34)***	***(+68)***	***(+35)***

**: $p \leq 0.01$,
PB: Phenobarbital
[a] % change compared to control

At 1500 ppm, enlarged liver was noted (in 19/20 males and in all females) as well as prominent lobulation on liver (9/20 males) and pale liver (11/20 females). Also at 500 ppm, enlarged liver was noted (14/20 males and 12/20 females) as well as pale liver (11/20 females).

Total cytochrome P-450 content was increased in a dose-related manner by between 36 % ($p \leq 0.05$, 25 ppm) and 282 % ($p < 0.01$, 1500 ppm) in males and by between 16 % (not statistically significant, 25 ppm) and 185 % ($p < 0.01$, 1500 ppm) in females. The highest enzymatic activity dose-related increases observed were PROD and BROD activities, starting at 25 ppm in the males and at 500 ppm in the females. EROD activity was slightly increased in both sexes from 500 ppm on. A slight increase in UGT2 activity was observed from 500 ppm in the

males. LAH activity was slightly decreased at 1500 ppm in the males.

Table 6.5-49. Total cytochrome P-450 content and enzymatic activities after 28-day exposure. Mean ± standard deviation (the means and standard deviation are calculated with 4 pools of 5 animals in each group)

	Tebuconazole [ppm]				**PB [mg/kg bw]**
	0	**25**	**500**	**1500**	**80**
Males					
P-450 [nmol/mg Prot.] *(%)[a]*	1.1 ± 0.2	**1.5 ± 0.1*** ***(+36)***	**3.2 ± 0.6**** ***(+183)***	**4.3 ± 0.4**** ***(+282)***	**2.8 ± 0.2**** ***(+147)***
EROD [pmol/min/ mg Prot.] *(%)[a]*	34.7 ± 3.9	44.3 ± 4.0 *(+27.4)*	**51.6 ± 4.6**** ***(+48.5)***	**131.8 ± 29.7**** ***(+279)***	**103.4 ± 15.9**** ***(+198)***
PROD [pmol/min/ mg Prot.] *(%)[a]*	3.7 ± 0.5	**8.0 ± 1.3**** ***(+116)***	**39.8 ± 1.9**** ***(+972)***	**54.9 ± 6.8**** ***(+1381)***	**131.2 ± 25.6**** ***(+3437)***
BROD [pmol/min/ mg Prot.] *(%)[a]*	7.7 ± 1.9	**31.5 ± 7.5**** ***(+312)***	**288.7 ± 18.4**** ***(+3669)***	**428.1 ± 52.1**** ***(+5489)***	**926.8 ± 130.9**** ***(+11999)***
UGT2 [pmol/min/ mg Prot.] *(%)[a]*	0.62 ± 0.06	0.79 ± 0.06 *(+27)*	**1.04 ± 0.27**** ***(+67)***	**1.09 ± 0.13**** ***(+75)***	**1.6 ±0.29**** ***(+158)***
LAH [nmol/min/ mg Prot.] *(%)[a]*	7.94	7.67 *(-3)*	6.63 *(-17)*	3.35 *(-58)*	5.28 *(-33.5)*
Females					
P-450 [nmol/mg Prot.] *(%)[a]*	1.4 ± 0.2	1.6 ± 0.04 *(+16)*	**3.2 ± 0.3**** ***(+129)***	**4.0 ± 0.4**** ***(+185)***	**3.0 ± 0.2**** ***(+115)***
EROD [pmol/min/ mg Prot.] *(%)[a]*	46.0 ± 7.1	50.8 ± 8.0 *(+10)*	**62.1 ± 9.2*** ***(+35)***	**99.7 ± 5.3**** ***(+517)***	**88.2 ± 13.8**** ***(+92)***
PROD [pmol/min/ mg Prot.] *(%)[a]*	10.0 ± 2.6	11.6 ± 1.6 *(+16)*	**42.0 ± 5.3**** ***(+319)***	**61.8 ± 6.6**** ***(+517)***	**134.2 ± 3.2**** ***(+1238)***
BROD [pmol/min/ mg Prot.] *(%)[a]*	25.5 ± 12.9	40.8 ± 12.6 *(+60)*	**356.5 ± 37.4**** ***(+1296)***	**517.5 ± 63.0**** ***(+1927)***	**932.9 ± 55.6**** ***(+3554)***
UGT2 [pmol/min/ mg Prot.] *(%)[a]*	1.70 ± 0.04	1.80 ± 0.18 *(+6)*	1.68 ± 0.48 *(-1)*	1.34 ± 0.11 *(-21)*	**3.33 ± 0.09**** ***(-96)***
LAH [nmol/min/ mg Prot.] *(%)[a]*	9.57	12.17 *(+27)*	6.22 *(-35)*	4.58 *(-52)*	4.05 *(-58)*

Values in bold and bold combined with italics are significantly different from control at $p \leq 0.05$ or $p \leq 0.01$

*: $p \leq 0.05$; **: $p \leq 0.01$;

PB = Phenobarbital

[a] % change compared to control

At 1500 ppm in the liver, the most highly up-regulated phase I enzyme gene transcripts, both in males and females, were Cyp 2B10 and Cyp 3A11 when compared to the controls. Cyp 2B9 gene transcripts were also strongly up-regulated in the male mice only whereas slightly down-regulated in the females. Cyp 4A10 gene transcripts were slightly down-regulated in both sexes. Regarding the phase II enzyme, Ugt1A1 gene transcripts were slightly up and down-regulated in males and females, respectively whereas Ugt2B1 gene transcripts were slightly down-regulated in male and female mice when compared to the controls. Growth arrest and DNA-damage-inducible protein GADD45 alpha (Gadd45a, increased following stressful growth arrest conditions) gene transcripts were

slightly down-regulated in the female mice. The peroxisomal Acox1 gene transcripts were slightly down-regulated in both sexes. At 500 ppm in the liver, the most highly up-regulated phase I gene transcripts, both in males and females, were Cyp 2B10 and Cyp 3A11. Cyp 2B9 gene transcripts were slightly down-regulated in the female mice only. Cyp 4A10 gene transcripts were slightly down-regulated in both sexes. The unique phase II enzyme gene transcripts deregulated were Ugt2B1, slightly down-regulated in the female mice only. Gadd45a gene transcripts were slightly down-regulated in both sexes when compared to the controls. At 25 ppm in the liver, Cyp 2B10 gene transcripts were up-regulated in the male mice.

Reference substance, phenobarbital:
A reduction of mean body weight due to a mean body weight loss and a cumulative body weight loss or weak gain) was observed in both sexes at 80 mg/kg bw/day. At necropsy, a lower mean terminal body weight (-6 %, $p \leq 0.05$ and -5 %, $p \leq 0.05$, in males and females, respectively) was statistically significant in both sexes when compared to the controls.

In females, mean food consumption was reduced by 16 % ($p \leq 0.01$, Study Day 8) compare to the controls.

Clinical chemistry analyses performed after 28-day exposure revealed treatment-related changes (lower total cholesterol and lower total protein and albumin in females, lower total bilirubin, and higher aminotransferases and alkaline phosphatase) in both sexes.

At 80 mg/kg bw for 28 days, mean absolute and relative liver weight were statistically significantly higher when compared to controls in both sexes and enlarged liver (in 10/20 males and in 15/20 females) as well as dark liver (14/20 males and 9/20 females) and white foci on liver (8/20 females) were noted.

Following exposure for 28 days, total cytochrome P-450 content was increased by 147 %) ($p \leq 0.01$) and by 115 % ($p \leq 0.01$) in males and females, respectively. The highest enzymatic activity increases observed were PROD and BROD activities, both in males and females. EROD and UGT2 activities were slightly increased in both sexes.

At 80 mg/kg/day for 28 days, in the liver, the most highly up-regulated phase I enzyme gene transcripts, both in males and females, were Cyp 2B10 and Cyp 3A11 when compared to the controls. Cyp 2B9 gene transcripts were also up-regulated in the male mice only whereas slightly down-regulated in the females. Cyp 4A10 gene transcripts were slightly down-regulated in both sexes. Regarding the phase II enzyme, UgtlAl gene transcripts were slightly up in males and females. The anti-apoptotic gene transcripts Bcl-Xl (females only) were slightly down-regulated. The peroxisomal Acoxl gene transcripts were slightly down-regulated in both sexes.

Conclusion

In conclusion, these results suggest that tebuconazole is not a peroxisome proliferator since neither an induction of LAH – Lauric Acid Hydroxylase (at 28-day exposure) nor an increase in Acoxl gene transcript (at the two periods of exposure) were observed after tebuconazole treatment in both sexes. In contrast, tebuconazole induced BROD and PROD activities as well as an increase in Cyp 2b and Cyp 3a gene transcripts (males > females) to a lesser extent than phenobarbital, indicating that CAR/PXR receptors were indeed activated by tebuconazole.

Previous evaluation	None – submitted for the purpose of renewal (study owned by Bayer Task force)

Study ID	B.6.5.3/02
Study title	Tebuconazole - 28-day liver mechanistic study in the male and female mice by dietary administration (liver histopathology and cell proliferation investigations)
Test substance	Tebuconazole
Purity (%) **Batch no.**	97.5 K689052
GLP	Yes
Guideline	n/a
Deviation	n/a
Acceptable	Acceptable as a mechanistic study

Methods
Tebuconazole was administered continuously via the diet to groups of NMRI mice (20/sex/group) for at least 7 or 28 days at concentrations of 0, 25, 500 and 1500 ppm (equating approximately to 4, 72 and 201 mg/kg bw/day in males and 5, 87 and 273 mg/kg bw/day in females for the 7-day exposure period and equating approximately to 4, 77 and 231 mg/kg bw/day in males and 5, 90 and 276 mg/kg bw/day in females for the 28-day exposure period) to investigate liver histopathology and cell proliferation. An additional group received Phenobarbital (suspension in 0.5 % aqueous solution of methylcellulose) by oral gavage once per day at 80 mg/kg/day and acted as positive control for enzyme induction. Clinical observations, body weights and food consumption were investigated throughout the duration of the study. All animals were subjected to necropsy, brain and liver were weighed and selected portions of the liver were fixed for conventional histopathological examination. Cell proliferation measurements following immunohistochemical staining were performed on liver sections using the Ki67 marker.

Results

7-day exposure
Mean body weight, mean body weight gain and the overall cumulative body weight were affected (reduction of mean body weight due to a mean body weight loss and a cumulative body weight loss) only at 1500 ppm in both sexes. At necropsy, at 1500 ppm in males, mean terminal body weight was statistically significantly lower (-10 %, $p \leq 0.01$) when compared to controls. At 1500 ppm, mean food consumption was reduced by 19 % ($p \leq 0.01$) in males only at Study Day 8 compared to the controls.

At 1500 ppm and 500 ppm, mean absolute and relative liver weights were statistically significantly higher when compared to controls in both sexes.

At 1500 ppm, enlarged livers were noted (in all animals) as well as prominent lobulation on liver (5/15 males and 3/15 females), pale livers (6/15 females), and white foci on liver (4/15 males). At 500 ppm, enlarged livers were noted (12/15 males and 14/15 females) as well as pale livers (7/15 females).

At 1500 ppm, microscopic examination revealed hepatocellular hypertrophy, single cell necrosis, micro/macrovacuolation, increased number of mitoses and interstitial mixed cell infiltrate in both sexes. Hepatocellular necrotic foci were also noted in males at 1500 ppm. At 500 ppm, microscopic examination revealed hepatocellular hypertrophy, single cell necrosis and micro/macrovacuolation in both sexes. Hepatocellular necrotic foci were also noted in males at 500 ppm.

Table 6.5-50. Incidence and severity of microscopic findings after 7-day exposure

	Males				Females			
Dose [ppm]	**0**	**25**	**500**	**1500**	**0**	**25**	**500**	**1500**
Number of animals examined	15	15	15	15	15	15	15	15
Hepatocellular hypertrophy: centrilobular to panlobular								
Slight	0	0	4	3	0	0	2	2
Moderate	0	0	11	11	0	0	12	13
Marked	0	0	0	1	0	0	1	0
Total	0	0	15	15	0	0	15	15
Hepatocellular single cell necrosis: focal								
Minimal	1	1	5	7	0	0	2	10
Slight	0	0	0	7	0	0	1	3
Moderate	1	1	0	1	0	0	0	0
Total	2	2	5	15	0	0	3	13
Hepatocellular necrotic focus(i): focal								
Minimal	0	1	5	1	1	2	1	0
Slight	0	0	0	2	0	0	0	1
Total	0	1	5	3	1	2	1	1
Interstitial mixed cell infiltrate: focal								
Minimal	0	1	0	3	0	0	1	6
Slight	0	0	0	1	0	0	0	0
Total	0	1	0	4	0	0	1	6

Dose [ppm]	Males 0	25	500	1500	Females 0	25	500	1500
Number of animals examined	15	15	15	15	15	15	15	15
Increased number of mitoses								
Present	0	0	0	5	0	0	0	4
Hepatocellular micro/macrovacuolation: diffuse								
Minimal	0	0	9	8	0	0	1	1
Slight	0	0	1	0	0	0	10	5
Moderate	0	0	0	1	0	0	4	8
Marked	0	0	0	0	0	0	0	1
Total	0	0	10	9	0	0	15	15

At 1500 ppm, assessment of cell proliferation in the liver revealed 24 and 57 times higher Ki67 labelling index in the centrilobular area in treated males and females, respectively, when compared to the controls. Assessment of cell proliferation in the periportal area of the liver revealed 5 and 26 times higher Ki67 labelling index in treated males and females, respectively, when compared to the controls. The overall Ki67 labelling index (centrilobular + periportal) was higher (approximately 13 and 43 times for the males and females, respectively) than the controls.

In females at 500 ppm, assessment of cell proliferation in the liver revealed 2.1 times higher Ki67 labelling index in the centrilobular area in treated females when compared to the controls. Assessment of cell proliferation in the periportal area of the liver revealed 2.8 times higher Ki67 labelling index in treated females when compared to the controls. The overall Ki67 labelling index (centrilobular + periportal) was higher 2.4 times for the females than the controls.

28-day exposure

Mean body weight, mean body weight gain and the overall cumulative body weight were affected (reduction of mean body weight due to a mean body weight loss in the first week and a cumulative body weight loss throughout the study period) only at 1500 ppm and only in males. At necropsy, at 1500 ppm in males, mean terminal body weight was statistically significantly lower (-8 %, $p \leq 0.01$) when compared to controls.

At 1500 ppm, mean food consumption was reduced by between 19 % ($p \leq 0.01$, Study Day 8) and 10 % ($p \leq 0.01$, Study Day 29) in males only compared to the controls.

At 1500 ppm and 500 ppm, mean absolute and relative liver weight were statistically significantly higher when compared to controls in both sexes. At 1500 ppm, enlarged livers were noted (in 14/15 animals) as well as prominent lobulation on liver (7/15 males), pale livers (6/15 females). At 500 ppm, enlarged livers were noted (13/15 males and 12/15 females) as well as pale livers (7/15 females).

Table 6.5-51. Organ weights

Dose [ppm]	Males 0	25	500	1500	Females 0	25	500	1500
7-day exposure								
Absolute liver weight [g]	1.41 ± 0.07	1.42 ± 0.10	**1.86**** **± 0.22**	**2.14**** **± 0.25**	1.09 ± 0.08	1.13 ± 0.10	**1.66**** **± 0.16**	**1.94**** **± 0.18**
(%)[a]	--	*(+1)*	***(+31)***	***(+52)***	--	*(+4)*	***(+52)***	***(+78)***
Liver / body weight ratio [%]	4.05 ± 0.21	4.07 ± 0.16	**5.37**** **± 0.42**	**6.84**** **± 0.53**	4.03 ± 0.24	4.13 ± 0.25	**6.03**** **± 0.59**	**7.30**** **± 0.57**
(%)[a]	--	*(±0)*	***(+33)***	***(+69)***	--	*(+2)*	***(+50)***	***(+81)***
Liver / brain weight ratio [%]	305.71 ± 18.85	299.46 ± 16.25	**395.96**** **± 47.69**	**464.34**** **± 49.34**	233.55 ± 23.98	236.08 ± 26.71	**354.29**** **± 35.64**	**426.68**** **± 38.94**
(%)[a]	--	*(-2)*	***(+30)***	***(+52)***	--	*(+1)*	***(+52)***	***(+83)***
28-day exposure								
Absolute liver weight [g]	1.42 ± 0.12	1.41 ± 0.11	**1.94**** **± 0.22**	**2.54**** **± 0.34**	1.08 ± 0.09	1.11 ± 0.08	**1.58**** **± 0.19**	**1.99**** **± 0.31**
(%)[a]	--	*(-1)*	***(+37)***	***(+79)***	--	*(+4)*	***(+52)***	***(+78)***
Liver / body	3.74	3.84	**5.25****	**7.24****	3.90	3.91	**5.57****	**7.12****

	Males				Females			
Dose [ppm]	**0**	**25**	**500**	**1500**	**0**	**25**	**500**	**1500**
weight ratio [%]	± 0.23	± 0.18	**± 0.50**	**± 0.81**	± 0.25	± 0.27	**± 0.53**	**± 0.92**
(%)[a]	--	*(+3)*	***(+41)***	***(+94)***	--	*(+2)*	***(+50)***	***(+81)***
Liver / brain	295.62	301.72	**412.78**[**]	**557.86**[**]	226.73	240.11	**335.91**[**]	**437.00**[**]
weight ratio [%]	± 25.93	± 29.65	**± 43.53**	**± 75.93**	± 18.44	± 20.70	**± 45.94**	**± 72.89**
(%)[a]	--	*(+2)*	***(+40)***	***(+89)***	--	*(+6)*	***(+48)***	***(+93)***

[**] statistically significantly different at $p \leq 0.01$
Values in bold and bold combined with italics are significantly different from control

Table 6.5-52. Gross pathology of liver, number of findings, n = 15

	Males				Females			
Dose [ppm]	**0**	**25**	**500**	**1500**	**0[#]**	**25**	**500**	**1500**
7-day exposure								
Enlarged	0	0	12	15	0	0	14	15
Prominent lobulation	0	0	0	5	0	0	0	3
Pale	0	0	1	0	0	0	6	7
Focus(i), white	0	0	1	4	0	0	1	0
28-day exposure								
Enlarged	0	0	13	14	0	0	12	14
Prominent lobulation	0	0	1	7	0	0	0	1
Pale	0	0	0	2	0	1	7	6

[#] Control group of the 28-day exposure animals consisted only of 14 animals

At 1500 ppm, microscopic examination revealed hepatocellular hypertrophy, single cell necrosis, micro/macrovacuolation, increased number of mitoses and interstitial mixed cell infiltrate in both sexes. Bile duct hyperplasia was also noted in males and intracanalicular cholestasis and accumulation of brown pigments in Kupffer cells were observed in females. At 500 ppm, microscopic examination revealed hepatocellular hypertrophy, single cell necrosis, micro/macrovacuolation and interstitial mixed cell infiltrate in both sexes.

Table 6.5-53. Incidence and severity of microscopic findings after 28-day exposure

	Males				Females			
Dose [ppm]	**0**	**25**	**500**	**1500**	**0**	**25**	**500**	**1500**
n	15	15	15	15	14	15	15	15
Hepatocellular hypertrophy: centrilobular to panlobular								
Minimal	0	1	0	0	0	0	1	0
Slight	0	0	7	1	0	0	12	2
Moderate	0	0	8	9	0	0	2	12
Marked	0	0	0	5	0	0	0	1
Total	0	1	15	15	0	0	15	15
Hepatocellular single cell necrosis: focal								
Minimal	0	0	7	0	0	0	5	2
Slight	0	0	1	7	0	0	1	10
Moderate	0	0	0	7	0	0	0	2
Total	0	0	8	14	0	0	6	14
Bile duct hyperplasia: focal								
Minimal	0	0	0	2	0	0	0	0
Interstitial mixed cell infiltrate: focal								
Minimal	1	2	5	8	1	2	7	5
Slight	0	0	0	0	0	0	0	1
Total	1	2	5	8	1	2	7	6
Increased number of mitoses								
Present	0	0	0	3	0	0	0	1
Hepatocellular micro/macrovacuolation: diffuse								

	Males				Females			
Dose [ppm]	**0**	**25**	**500**	**1500**	**0**	**25**	**500**	**1500**
n	15	15	15	15	14	15	15	15
Minimal	0	0	6	2	0	0	2	1
Slight	0	0	7	3	0	0	10	6
Moderate	0	0	0	5	0	0	3	5
Marked	0	0	0	0	0	0	0	2
Total	0	0	13	10	0	0	15	14
Cholestasis: intracanalicular								
Minimal	0	0	0	0	0	0	0	4
Accumulation of brown pigment in Kupffer cells								
Minimal	0	0	0	0	0	0	0	2

At 1500 ppm, assessment of cell proliferation in the liver revealed 24 and 18 times higher Ki67 labelling index in the centrilobular area in treated males and females, respectively, when compared to the controls. Assessment of cell proliferation in the periportal area of the liver revealed 38 and 21 times higher Ki67 labelling index in treated males and females, respectively, when compared to the controls. The overall Ki67 labelling index (centrilobular + periportal) was higher (approximately 30 and 19 times for the males and females, respectively) than the controls.

Table 6.5-54. Cell cycling assessment (mean ± standard deviation)

	Males				Females			
Dose [ppm]	**0**	**25**	**500**	**1500**	**0**	**25**	**500**	**1500**
7-day exposure								
n	15	15	15	14	15	14	15	15
Centrilobular	2.08 ± 5.02	0.41 ± 0.49	2.81 ± 3.36	**49.90**** **± 45.54**	1.98 ± 1.51	1.67 ± 1.77	4.10 ± 5.10	**113.04**** **± 41.85**
Periportal	3.10 ± 6.74	0.67 ± 1.44	0.70 ± 0.72	**15.44**** **± 16.84**	1.67 ± 1.29	1.00 ± 1.36	**4.67*** **± 6.03**	**43.30**** **± 20.51**
Total	2.59 ± 5.85	0.54 ± 0.88	1.76 ± 1.77	**32.67**** **± 28.83**	1.83 ± 0.94	1.33 ± 1.21	4.39 ± 5.16	**78.17**** **± 27.01**
28-day exposure								
n	15	15	15	15	14	15	15	15
Centrilobular	0.68 ± 0.89	1.06 ± 1.16	1.82 ± 2.38	**16.58**** **± 15.90**	1.43 ± 2.15	1.55 ± 1.68	1.67 ± 1.80	**25.77**** **± 28.24**
Periportal	0.45 ± 0.68	**1.74**** **± 1.49**	0.87 ± 0.98	**17.13**** **± 23.29**	0.95 ± 1.14	1.07 ± 1.09	2.02 ± 2.60	**19.73**** **± 14.24**
Total	0.56 ± 0.54	**1.40**** **± 1.09**	1.35 ± 1.56	**16.85**** **± 17.24**	1.19 ± 1.58	1.31 ± 1.05	1.84 ± 1.94	**22.75**** **± 19.40**

* / ** statistically significantly different at $p \leq 0.05$ / $p \leq 0.01$ respectively

Conclusion

In conclusion, 1500 ppm of tebuconazole induced marked liver cytotoxicity and cell proliferation throughout the study, for both the 7- and 28-day exposure periods. At 500 ppm liver cytotoxicity (hepatocellular single cell necrosis) was noted in both sexes for both the 7- and 28-day exposure periods, as well as a slight cell proliferation in females only for the 7-Day period. No effect on the liver was observed at the lowest dose level investigated (25 ppm) for the parameters examined.

Previous evaluation	None – submitted for the purpose of renewal (study owned by Bayer Task force)

Study ID	B.6.5.3/03
Study title	Tebuconazole - DNA-synthesis induction in cultured male C57BL/6 mouse hepatocytes
Test substance	Tebuconazole
Purity (%)	98.2

Batch no.	95893115
GLP	Yes
Guideline	n/a
Deviation	n/a
Acceptable	Acceptable as a mechanistic study

Methods

The aim of this study was to investigate the potential for tebuconazole to stimulate hepatocellular proliferation (measured as the change in replicative DNA synthesis [S-phase of the cell cycle]) in isolated male C57BL/6 wild type (WT) mouse hepatocyte cultures. Adenosine 5′-triphosphate (ATP) depletion as a measure of cytotoxicity was assessed in parallel. Phenobarbital sodium salt (PB) and epidermal growth factor (EGF) served as positive control substances.

Based on a preliminary dose range finding study, primary monolayer cultures of hepatocytes were prepared and exposed to tebuconazole at 4 concentrations (1, 3, 10 and 30 µM) or to a vehicle control (0.1% DMSO) for 96 hours. Additional hepatocytes were exposed to PB at 1000 µM for 96 hours as a positive control. EGF was tested at a single concentration (25 ng/mL) for 96 hours as a positive control agent for replicative DNA synthesis.

Results

Tebuconazole reduced ATP levels by 23 % in the male C57BL/6 WT mouse hepatocyte culture at the highest concentration assessed (30 µM). Small reductions (< 20 %) in ATP levels were observed after treatment with either 1 µM tebuconazole or 1000 µM PB, but these decreases were not considered biologically relevant.

Table 6.5-55. ATP Assay following tebuconazole or PB administration

Test/Control Item & concentration	ATP content (luminescence units)
Vehicle control (0.1% [v/v] DMSO)	404193 ± 21354[a] (100.0 ± 5.3)
PB 1000 µM	328284 ± 35292 (81.2 ± 8.7)**
Tebuconazole 1 µM	404177 ± 15874 (100.0 ± 3.9)
Tebuconazole 3 µM	335462 ± 50055 (83.0 ± 12.4)*
Tebuconazole 10 µM	414763 ± 13414 (102.6 ± 3.3)
Tebuconazole 30 µM	310314 ± 36154 (76.8 ± 8.9)***

[a] Values are Mean ± SD, Values in parenthesis are mean % control ± SD
n=6 per group
Student's t-test (2-tailed) : * statistically different from control $p<0.05$; ** $p<0.01$; *** $p<0.001$

Treatment with tebuconazole caused concentration-dependent, statistically-significant increases in replicative DNA synthesis as determined by the S-phase labelling index, to a maximum of 1.7-fold at 3 µM. Replicative DNA synthesis levels then decreased at 30 µM to 1.3-fold, a concentration at which cytotoxicity was seen in the form of ATP. As expected, treatment with PB or EGF resulted in statistically-significant increases in replicative DNA synthesis of 1.8- and 8.5- fold, respectively.

Table 6.5-56. Replicative DNA synthesis (S-Phase) assessment following tebuconazole, PB or EGF administration

Test/Control Item & concentration	S-phase labelling index
Vehicle control (0.1% [v/v] DMSO)	0.51 ± 0.06[a] (100.0 ± 11.7)
PB 1000 µM	0.92 ± 0.15 (178.8 ± 29.3)***
Tebuconazole 1 µM	0.54 ± 0.07 (105.9 ± 13.7)
Tebuconazole 3 µM	0.88 ± 0.12 (172.5 ± 24.1)***
Tebuconazole 10 µM	0.84 ± 0.06 (163.0 ± 12.4)***
Tebuconazole 30 µM	0.68 ± 0.18 (132.1 ± 34.2)
EGF 25 ng/mL	4.34 ± 0.66 (846.1 ± 128.1)***

[a] Values are Mean ± SD, Values in parenthesis are mean % control ± SD
n=5 per group
Student's t-test (2-tailed): *** statistically different from control $p<0.001$

Conclusion

Overall, treatment of isolated male C57BL/6 WT mouse hepatocyte cultures with tebuconazole resulted in concentration-dependent increases in replicative DNA synthesis as determined by the S-phase labelling index, which peaked at 3 µM. Treatment with the positive control items PB and EGF gave the expected set of responses, indicating the suitability of the test system.

Previous evaluation	None – submitted for the purpose of renewal (study owned by Bayer Task force)

Study ID	B.6.5.3/04
Study title	Tebuconazole - DNA-synthesis induction in cultured male CarKO/PxrKO mouse hepatocytes
Test substance	Tebuconazole
Purity (%) **Batch no.**	98.2 95893115
GLP	Yes
Guideline	n/a
Deviation	n/a
Acceptable	Acceptable as a mechanistic study

Methods

The aim of this study was to investigate the potential for tebuconazole to stimulate hepatocellular proliferation (measured as the change in replicative DNA synthesis [S-phase of the cell cycle]) in isolated male constitutive androstane receptor knockout/pregnane x receptor knockout (CarKO/ PxrKO) mouse hepatocyte cultures. Adenosine 5′-triphosphate (ATP) depletion as a measure of cytotoxicity was assessed in parallel. Phenobarbital sodium salt (PB) and epidermal growth factor (EGF) served as control substances.

Based on a preliminary dose range finding study, primary monolayer cultures of hepatocytes were isolated and exposed to tebuconazole at 4 concentrations (1, 3, 10 and 30 µM) or to a vehicle control (0.1% DMSO) for 96 hours. Additional hepatocytes were exposed to PB at 1000 µM as a control for 96 hours. There were 5 replicates for each concentration for replicative DNA synthesis (incorporation of 5-bromo-2′-deoxyuridine [BrdU]) and 6 replicates for each concentration for cytotoxicity measurements (measured as the change in cellular ATP). EGF was tested at a single concentration (25 ng/mL) for 96 hours as a positive control agent for replicative DNA synthesis.

Results

At all concentrations assessed, tebuconazole did not cause cytotoxicity, as shown by the small reductions in ATP levels (by < 10 %) in the male CarKO/PxrKO mouse hepatocyte cultures. Although treatment with PB increased ATP levels slightly compared with control, this increase was not considered biologically relevant.

Table 6.5-57. ATP assay following tebuconazole or PB administration

Test/Control Item & concentration	ATP content (luminescence units)
Vehicle control (0.1% [v/v] DMSO)	451179 ± 7320[a] (100.0 ±1.6)
PB 1000 µM	462988 ± 8173 (102.6 ± 1.8)*
Tebuconazole 1 µM	432593 ± 10762 (95.9 ± 2.4)**
Tebuconazole 3 µM	411740 ± 7598 (91.3 ± 1.7)***
Tebuconazole 10 µM	424230 ± 10022 (94.0 ± 2.2)***
Tebuconazole 30 µM	411918 ± 9758 (91.3 ± 2.2)***

[a] Values are Mean ± SD, Values in parenthesis are mean % control ± SD
n=6 per group
Student's t-test (2-tailed): * statistically different from control $p<0.05$; ** $p<0.01$; *** $p<0.001$

Treatment with either tebuconazole or PB failed to increase replicative DNA synthesis as determined by S-phase labelling index. As expected, treatment with EGF resulted in a statistically-significant increase in replicative DNA synthesis of 3.8- fold.

Table 6.5-58. Replicative DNA synthesis (S-Phase) assessment following tebuconazole, PB or EGF administration

Test/Control Item & concentration	S-phase labelling index
Vehicle control (0.1% [v/v] DMSO)	0.45 ± 0.06[a] (100.0 ± 14.0)
PB 1000 µM	0.38 ± 0.04 (85.6 ± 8.0)
Tebuconazole 1 µM	0.43 ± 0.05 (95.8 ± 12.1)
Tebuconazole 3 µM	0.53 ± 0.11 (119.4 ± 24.1)
Tebuconazole 10 µM	0.41 ± 0.06 (93.1 ± 12.4)
Tebuconazole 30 µM	0.41 ± 0.06 (92.8 ± 12.7)
EGF 25 ng/mL	1.71 ± 0.08 (384.6 ± 17.9)***

[a] Values are Mean ± SD, Values in parenthesis are mean % control ± SD
n=5 per group
Student's t-test (2-tailed): *** statistically different from control $p<0.001$

Conclusion

There was no increase in replicative DNA synthesis following treatment of isolated male CarKO/PxrKO mouse hepatocyte cultures with either tebuconazole or PB, as determined by S-phase labelling index. Treatment with the

positive control item EGF gave the expected response, indicating the suitability of the test system.

Previous evaluation	None – submitted for the purpose of renewal (study owned by Bayer Task force)

Study ID	B.6.5.3/05
Study title	Tebuconazole - DNA-synthesis induction in cultured male human hepatocytes from three individual donors
Test substance	Tebuconazole
Purity (%) **Batch no.**	98.2 95893115
GLP	Yes
Guideline	n/a
Deviation	n/a
Acceptable	Acceptable as a mechanistic study

Methods

The aim of this study was to investigate the potential for tebuconazole to stimulate hepatocellular proliferation (measured as the change in replicative DNA synthesis [S-phase of the cell cycle]) in male human hepatocyte cultures from 3 individual human donors. Adenosine 5′-triphosphate (ATP) depletion as a measure of cytotoxicity was assessed in parallel. Phenobarbital sodium salt (PB) and epidermal growth factor (EGF) served as control substances.

Treatment with 300 and 500 µM tebuconazole resulted in < 10 % cell viability compared with control ATP levels in all three individual male human primary hepatocyte donors in a preliminary study (Chatham, 2017). A biologically significant reduction in ATP levels (20-50% compared to control) was also observed after treatment with 100 µM tebuconazole. Therefore, it was decided that 50 µM would be the top concentration assessed in the current study. Primary monolayer cultures of hepatocytes were prepared from each individual donor and exposed to tebuconazole at 4 concentrations (3, 10, 30 and 50 µM) or to a vehicle control (0.1% DMSO) for 96 hours. Additional hepatocytes were exposed to PB at 1000 µM as a control for 96 hours. There were 5 replicates for each concentration for replicative DNA synthesis (incorporation of 5-bromo-2′-deoxyuridine [BrdU]) and 6 replicates for each concentration for cytotoxicity measurements (measured as the change in cellular ATP). EGF was tested at a single concentration (25 ng/mL) for 96 hours as a positive control agent for replicative DNA synthesis.

Results

There was no evidence of tebuconazole-mediated cytotoxicity in any donor, as ATP levels were only reduced by 10 - 20 % compared with control at the highest concentration assessed (50 µM).

Table 6.5-59. ATP assay following tebuconazole or PB administration

Test/Control Item & concentration	ATP content (luminescence units) Donor 8210	ATP content (luminescence units) Donor 8219	ATP content (luminescence units) Donor 385
Vehicle control (0.1% [v/v] DMSO)	313346 ± 20166[a] (100.0 ± 6.4)	260706 ± 7923[a] (100.0 ± 3.0)	592905 ± 40717[a] (100.0 ± 6.9)
PB 1000 µM	304488 ± 38396 (97.2 ± 12.3)	321313 ± 26644 (123.2 ± 10.2)***	632184 ± 40680 (106.6 ± 6.9)
Tebuconazole 3 µM	362175 ± 37198 (115.6 ± 11.9)*	290970 ± 20678 (111.6 ± 7.9)**	614329 ± 82532 (103.6 ± 13.9)
Tebuconazole 10 µM	367831 ± 34825 (117.4 ± 11.1)**	306773 ± 30216 (117.7 ± 11.6)**	611993 ± 43464 (103.2 ± 7.3)
Tebuconazole 30 µM	302198 ± 30351 (96.4 ± 9.7)	285005 ± 26045 (109.3 ± 10.0)	585994 ± 47601 (98.8 ± 8.0)
Tebuconazole	250910 ± 21748	218753 ± 24661	535453 ± 56883

50 µM	(80.1 ± 6.9)***	(83.9 ± 9.5)**	(90.3 ± 9.6)

[a] Values are Mean ± SD, Values in parenthesis are mean % control ± SD
n=6 per group
Student's t-test (2-tailed): * statistically different from control $p<0.05$; ** $p<0.01$; *** $p<0.001$

As expected, treatment with EGF caused statistically-significant increases in replicative DNA synthesis in the three donors of 6.1-, 3.5- and 4.3- fold, respectively. Treatment with either tebuconazole or PB failed to increase replicative DNA synthesis as determined by the S-phase labelling index.

Table 6.5-60. Replicative DNA synthesis (S-Phase) assessment following tebuconazole, PB or EGF administration

Test/Control Item & concentration	S-phase Labelling Index Donor 8210	S-phase Labelling Index Donor 8219	S-phase Labelling Index Donor 385
Vehicle control (0.1% [v/v] DMSO)	0.20 ± 0.06[a] (100.0 ± 28.7)	0.39 ± 0.09 (100.0 ± 22.8)	0.50 ± 0.03 (100 ± 6.4)
PB 1000 µM	0.26 ± 0.04 (129.5 ± 21.5)	0.30 ± 0.06 (78.5 ± 16.3)	0.56 ± 0.07 (112.2 ± 13.5)
Tebuconazole 3 µM	0.22 ± 0.05 (109.4 ± 25.5)	0.38 ± 0.08 (97.3 ± 19.9)	0.49 ± 0.03 (99.3 ± 6.0)
Tebuconazole 10 µM	0.24 ± 0.08 (119.1 ± 40.1)	0.38 ± 0.06 (98.4 ± 15.4)	0.51 ± 0.08 (101.4 ± 15.8)
Tebuconazole 30 µM	0.22 ± 0.05 (107.7 ± 26.6)	0.40 ± 0.04 (103.8 ± 9.4)	0.57 ± 0.07 (113.9 ± 13.2)
Tebuconazole 50 µM	0.18 ± 0.07 (86.8 ± 32.9)	0.38 ± 0.10 (99.0 ± 24.8)	0.51 ± 0.13 (102.6 ± 25.2)
EGF 25 ng/mL	1.25 ± 0.11 (611.5 ± 55.8)***	1.37 ± 0.13 (353.3 ± 34.5)***	2.16 ± 0.20 (433.6 ± 39.6)***

[a] Values are Mean ± SD, Values in parenthesis are mean % control ± SD
n=5 per group
Student's t-test (2-tailed) *** statistically different from control $p<0.001$

Conclusion

There was no increase in replicative DNA synthesis following treatment of male primary human hepatocyte cultures from three individual donors with either tebuconazole or PB, as determined by the S-phase labelling index.

Treatment with the positive control item EGF gave the expected set of responses, indicating the suitability of the test system.

(Chathman, 2017c)

Previous evaluation	None – publication submitted for the purpose of renewal (study owned by Bayer Task force)

Study ID	B.6.5.3/06
Study title	Dose-response involvement of constitutive androstane receptor in mouse liver hypertrophy induced by triazole fungicides
Test substance	Tebuconazole
Purity (%) **Batch no.**	97.3 Not specified
GLP	No
Guideline	n/a
Deviation	n/a
Acceptable	Acceptable as a mechanistic study

Methods

To clarify the dose–response relationship between constitutive androstane receptor (CAR) activity, induction of cytochrome P450 2B (CYP2B) expression and hypertrophy by triazole fungicides in mouse liver, three dose levels of cyproconazole (Cypro), tebuconazole (Teb), fluconazole (Flu), and phenobarbital (PB), a typical CYP2B inducer, were administrated in diet to male wild-type (WT) and CAR-knockout (CARKO) C3H/HeNCrl mice for one week. Focussing on tebuconazole, the test substance was administered at 0, 375, 750 or 1500 ppm (equivalent to 0, 74, 104, 209 mg/kg bw/day in WT mice and 0, 92, 150, 203 mg/kg bw/day in CARKO mice). PB was administered at 250, 500 or 1000 ppm (equivalent to 58, 81, 205 mg/kg bw/day in WT mice and 59, 95, 170 mg/kg bw/day in CARKO mice). Clinical observations, body weights and food consumption were monitored throughout the study. At termination, blood samples were collected and clinical-chemistry parameters were analysed. Liver weights were measured and histopathology performed. Expression of CYPs in the liver was measured using immunohistochemical staining and hepatocyte cell proliferation was analysed by counting the number of PCNA-positive cells.

Results

No treatment-related clinical signs indicating systemic toxicity were detected. The final body weights in the high-dose tebuconazole group of both mouse genotypes were lower than the corresponding control groups.

The relative liver weights were significantly and dose-dependently increased with Teb and PB at all doses in WT mice. These increases were accompanied by hepatocellular hypertrophy and CYP2B expression. In CARKO mice, increased liver weights, hypertrophy and CYP2B expression were also observed with Teb at all doses, but these were less marked than in WT mice. However, no increases in liver weight, no hypertrophy and no CYP2B expression were detected for all PB doses in CARKO mice. In addition, vacuolation or single cell necrosis in the hepatocytes and inflammatory cell infiltration were detected in both mouse genotypes exposed to Teb, but these findings were not observed in both mouse genotypes treated with PB.

ALT levels were dose-dependently increased in WT mice at the middle and high doses of Teb. However, in CARKO mice, a significant increase in ALT levels was only seen at the high dose of Teb. ALT levels were not affected in both mouse genotypes treated with PB.

In WT mice, hepatocyte proliferation was increased at the middle and high dose of Teb and PB. However, no increase was seen in CARKO mice treated with either Teb or PB.

Conclusion

The results of this study indicate that while CAR activation is fully responsible for the liver hypertrophy, CYP2B induction and hepatocyte proliferation caused by PB in the mouse, CAR activation is not solely responsible for the equivalent liver effects caused by Teb in the mouse. The data suggest that for Teb non-CAR routes, in particular PXR activation, are also important in causing liver hypertrophy, CYP2B induction and hepatocyte proliferation in the mouse.

Previous evaluation	None – publication submitted for the purpose of renewal (study owned by Bayer Task force)

Study ID	B.6.5.3/07
Study title	Involvement of constitutive androstane receptor in liver hypertrophy and liver tumour development induced by triazole fungicides
Test substance	Tebuconazole
Purity (%) **Batch no.**	97.3 Not specified
GLP	No
Guideline	n/a
Deviation	n/a
Acceptable	Acceptable as a mechanistic study

Methods

The involvement of constitutive androstane receptor (CAR) activation in triazole-induced liver hypertrophy and tumorigenesis using CAR-knockout (CARKO) mice was investigated. Seven-week-old male CARKO and wild-type (WT) C3H/HeNCrl mice were treated with cyproconazole (Cypro), tebuconazole (Teb), or fluconazole (Flu) in the diet for 27 weeks after initiation by diethylnitrosamine (DEN). Focussing on tebuconazole, the test substance was administered to groups of 25 males at 0, or 1500 ppm (equivalent to 0, 226-336 mg/kg bw/day). Clinical observations, body weights and food consumption were monitored throughout the study. After 4, 13, or 27 weeks of treatment, blood from all mice was withdrawn to measure ALT and all animals were sacrificed. At weeks 4, 13 and 27, the livers were weighed and subject to histopathology. At week 4, expression of CYP2B in the liver was measured using immunohistochemical staining and hepatocyte cell proliferation was analysed by counting the number of PCNA-positive cells. In addition, gene transcripts of a number of enzymes and proteins were analysed by PCR.

Results

No treatment-related clinical signs were detected throughout the treatment period. Body weights were continuously reduced in mice of both genotypes treated with Teb. At week 27, the degrees of bodyweight reduction in WT and CARKO mice were about 13% and 18%, respectively. Food consumption in the WT mice was consistently low (−18.6% on average), while that of the CARKO mice was comparable to the control group value.

In the both the WT and CARKO mice treated with Teb, absolute and relative liver weights were significantly increased at weeks 4 and 13. Marked liver hypertrophy was observed in both the WT and CARKO mice treated with Teb.

Vacuolation of hepatocytes and infiltration of inflammatory cells were observed from week 4, while oval cell hyperplasia was observed at week 27 for both genotypes treated with Teb. Serum ALT levels were increased in both the WT and CARKO mice treated with Teb at all timepoints.

Hepatocyte cell proliferation was increased in both the WT and CARKO mice treated with Teb.

At week 4, CYP2B activity was increased in both genotypes treated with Teb compared to controls. Similarly, *Cyp2b10, Cyp3a11* and *Cyp4a10* expression was increased in both WT and CARKO mice treated with Teb compared to controls.

Although *Cyp1a2* and *Cyp4a10* expression was slightly but significantly increased in both genotypes treated with Teb, the expression was lower in CARKO mice compared toWT mice. Similarly, *P450 reductase* expression was significantly higher in both genotypes treated with Teb compared to controls; however, the increase inWT mice was higher than the corresponding increase in CARKO mice.
At week 27, Teb significantly increased the incidence of eosinophilic altered foci and/or adenomas inWT mice. However, these proliferating lesions were clearly reduced in CARKO mice treated with Teb.

Conclusions

The results of this study suggest that for Teb non-CAR routes, in particular PXR activation, are also important in causing liver hypertrophy, CYP2B induction and hepatocyte proliferation in the mouse. However, CAR activation seems essential in Teb-induced mouse liver tumour development.

Previous evaluation:	None – publication submitted for the purpose of renewal (study owned by Bayer Task force)

Study ID	B.6.5.3/08
Study title	CYP1A1 induction and CYP3A4 inhibition by the fungicide imazalil in the human intestinal Caco-2 cells-Comparison with other conazole pesticides
Test substance	Tebuconazole
Purity (%) **Batch no.**	Not specified Not specified
GLP	No
Guideline	n/a

Deviation	n/a
Acceptable	Acceptable as a mechanistic study

In the present study, the effect of four conazole-fungicides, two imidazole-derivatives, i.e. imazalil and ketoconazole, and two triazoles, i.e. propiconazole and tebuconazole, on the CYP1A1 activity in human intestinal Caco-2 cells was tested. An inducing effect on the CYP1A1 activity after treatment with the selected azoles was observed, imazalil being the most potent inducer. Imazalil revealed to be a CYP1A1 inducer as potent as B(a)P and TCDD. Tebuconazole also induced the CYP1A1 activity, but to a much lesser extent than imazalil.

B.6.5.4. Assessment of mode of action and human relevance of the mouse liver tumours

Introduction

Two mouse oncogenicity studies were conducted with tebuconazole. In the initial study (B.6.5.2/01), no treatment-related oncogenic effects were observed following treatment of male and female mice up to a concentration of 180 ppm (53/81 mg/kg bw/day in M/F) for up to 21 months. In a follow-up oncogenicity study in mice (B.6.5.2/02) with dietary concentrations of 0, 500 or 1500 ppm over a period of 21 months an increased incidence of liver adenomas and carcinomas in both sexes at the highest dose of 1500 ppm (280/357 mg/kg bw/day in M/F) was observed. An overview is given in the following table.

Table 6.5-61. Liver tumours findings in the second mouse oncogenicity study (B.6.5.2/02)

Parameter	Control data		Low dose 500 ppm		High dose 1500 ppm	
	m	f	m	f	m	f
Neoplastic changes						
Hepatocellular adenoma	3/47	0/47	2/48	0/45	17/48***	2/46
Hepatocellular carcinoma	0/47	1/47	0/48	0/45	10/48***	12/46***

* $p<0.05$, ** $p<0.01$, *** $p<0.001$
m: males; f: females

In a carcinogenicity study in rats in the same laboratory, no increases in liver tumour incidences were noted.

Proposed mode of action (MoA) and human relevance assessment

The weight of evidence indicates that the most likely MoA for the liver tumours seen in the mouse involves activation of the constitutive androstane receptor and/or pregnane X receptor (CAR/PXR). The key events involved are summarized in the following table.

Table 6.5-62. Listing of key events and associative events for a CAR/PXR-mediated liver tumour MoA

Events	Description
Key events (KE)	
KE 1	Activation of CAR/PXR nuclear receptor
KE 2	Altered gene expression secondary to CAR/PXR activation
KE 3	Increased hepatocellular proliferation
KE 4	Increased clonal expansion, leading to altered foci
KE 5	Increased incidence of hepatocellular tumours
Associative events (AE)	
AE 1	Increased Cyp2b, Cyp3a enzyme activity and/or protein
AE 2	Hepatocellular hypertrophy
AE 3	Increased liver weight

The mechanistic and regulatory studies described above have demonstrated these key events for tebuconazole.

Tebuconazole induced BROD and PROD activities as well as an increase in Cyp2b and Cyp3a gene transcripts in the available *in vivo* studies (KE 1, 2 and AE 1) which indicates that CAR/PXR receptors were activated by tebuconazole with subsequently altered gene expression. These key events together with increased hepatocellular proliferation (KE 3) were demonstrated *in vivo* (B.6.5.3/01). This was also supported by two publications (B.6.5.3/06, B.6.5.3/07) about triazole fungicides, including tebuconazole, investigating effects on the liver in wildtype (WT) and CAR knockout (CARKO) mice. Overall, these studies confirmed an involvement of the CAR and PXR receptors in the development of the tumours by showing fewer key events in CARKO mice treated with tebuconazole compared to WT mice treated with tebuconazole; also fewer altered hepatocellular foci occurred in CARKO mice than in WT mice treated with tebuconazole. The other key and associative events, like hepatocellular hypertrophy (AE 2), increased liver weight (AE 3) and eventually liver tumours (KE 5) were seen in the standard toxicology studies.

In addition to the *in vivo* mechanistic studies, *in vitro* studies were conducted with wild-type (WT) mouse (B.6.5.3/03), CAR/PXR-knockout (CarKO/PxrKO) mouse (B.6.5.3/04) and human (B.6.5.3/05) hepatocyte cultures exposed to tebuconazole. These studies confirmed the involvement of CAR/PXR in the postulated MoA since they demonstrated that hepatocyte proliferation was induced in WT mouse hepatocytes but not in CarKO/PxrKO mouse hepatocytes. Human hepatocytes did not show proliferation which clearly confirms that human hepatocytes are not sensitive to this MoA as the key event of hepatocyte proliferation does not occur in humans. On this basis, the postulated MoA is not relevant to humans.

Based on the available studies, other potential MoAs can also be excluded. A genotoxic MoA can be excluded based on the results of the genotoxicity studies which did not indicate a genotoxic potential for tebuconazole. Furthermore, neither an induction of LAH -Lauric Acid Hydroxylase (at 28-day exposure) nor an increase in Acoxl gene transcript (at 7- and 28-day exposure), two markers of peroxisome proliferation, was observed after treatment with tebuconazole, which suggests that tebuconazole is not a peroxisome proliferator. Also an effect on apoptosis is not likely based on the absence of clear effects on the apoptosis gene transcripts Bax and Bcl-X1. The negative Cyp1a1 results also indicate that there is no involvement of the AhR receptor.

Furthermore, severe liver cytotoxicity alone as a MoA for the liver tumours can be excluded, since in the toxicity studies with tebuconazole in mice at the highest dose tested, no signs of severe cytotoxicity in the liver, like inflammatory signs, broad hepatic necrosis, hepatocellular death, fibrosis, cirrhosis or severely increased transaminase activities were observed.

Overall, it can be concluded that the available evidence shows that the liver tumours seen in mice at the high dose of 1500 ppm (280/357 mg/kg bw/day in M/F) are most likely to arise through activation of CAR/PXR receptors, with consequent altered gene expression, hypertrophy, and hepatocyte proliferation, leading to altered foci and eventually tumours. The available evidence also shows that hepatocyte proliferation does not occur in human hepatocyte cultures exposed to tebuconazole, which clearly confirms that human hepatocytes are not sensitive to this MoA. On this basis, the postulated MoA and resulting liver tumours are not relevant to humans.

B.6.5.5. Summary of combined chronic toxicity and carcinogenicity

Three guideline oral combined chronic toxicity and carcinogenicity studies were described in the original DAR (2006), one in the rat and two in the mouse. All were conducted according to GLP and OECD test guidelines (available at the time the study was conducted) and are considered to be acceptable.

The following key conclusions were obtained from the evaluation of the long-term toxicity and carcinogenicity information:

- Tebuconazole did not show a carcinogenic potential of relevance to humans in rats or mice.
- Classification for carcinogenicity is not required
- The data requirements of Regulation 283/2013 have been met.

Study	Dose range tested (mg/kg bw/d)	NOAEL	LOAEL	Effects at the LOAEL	Study reference
In rats					

Study	Dose range tested (mg/kg bw/d)	NOAEL	LOAEL	Effects at the LOAEL	Study reference
Chronic/Carcinogenicity Oral/diet 2 year Tebuconazole Mixed batch Fl. no.: 132 Purity: > 95 % Rat Wistar Bor:WISW (SPF-Cpb) Male and female 50+10/sex/group GLP OECD test guideline no. 453 (1981)	0, 5.3, 15.9 and 55.0 (M) 0, 7.4, 22.8 and 86.3 (F)	Carcinogenicity: 1000 ppm (55 and 86 mg/kg bw/d for males and females respectively (top dose) Systemic toxicity: 300 ppm (23 mg/kg bw/d for females) 1000 ppm (55 mg/kg bw/d for males)	Carcinogenicity: - Systemic toxicity: 1000 ppm (86 mg/kg bw/d for females) (top dose) >1000 ppm (> 55 mg/kg bw/d for males)	Carcinogenicity: No treatment-related carcinogenic effects. Systemic effects: Lower body weight gain in females and histopathological findings in the adrenal, spleen and liver of females; No significant systemic toxicity in males	B.6.5.1/01
In mice					
Chronic/Carcinogenicity Oral/diet 21 months Tebuconazole Mixed batch Fl. no.: 132 Purity: > 95 % Mouse Bor:NMRI (SPF-Han) Male and female 50+10/sex/group GLP OECD test guideline no. 453 (1981)	0, 5.9, 18.2 and 53.1 (M) 0, 9.0, 26.1 and 80.5 (F)	Carcinogenicity: 180 ppm (53 and 81 mg/kg bw/d for males and females respectively (top dose) Systemic toxicity: 20 ppm (6 and 9 mg/kg bw/d for males and females respectively)	Carcinogenicity: - Systemic toxicity: 60 ppm (18 and 26 mg/kg bw/d for males and females respectively)	Carcinogenicity: No treatment-related carcinogenic effects. Systemic toxicity: Fatty degeneration/vacuolation of the liver in both sexes and increases in bilirubin in females.	B.6.5.2/01
Chronic/Carcinogenicity Oral/diet 21 months Tebuconazole Batch no. 816896061 Purity: 96.2 %	0, 85 and 279 (M) 0, 103, and 357 (F)	Carcinogenicity: 500 ppm (85 and 103 mg/kg bw/d for males and females respectively)	Carcinogenicity: 1500 ppm (280 and 357 mg/kg bw/d for males and females respectively) (top dose)	Carcinogenicity: Increased liver tumours in both sexes.	B.6.5.2/02

Study	Dose range tested (mg/kg bw/d)	NOAEL	LOAEL	Effects at the LOAEL	Study reference
Mouse NMRI Male and female 50+10/sex/group GLP OECD test guideline no. 453 (1981)		Systemic toxicity: -	Systemic toxicity: 500 ppm (85 and 103 mg/kg bw/d for males and females respectively)	Systemic effects: Liver toxicity and changes in some clinical-chemistry and haematological parameters.	

Rat
Administration of the test item produced a range of non-neoplastic treatment-related effects at the highest dose and only in females. At the top dose (1000 ppm – estimated to be 55 mg/kg bw/d in M and 86.3 mg/kg bw/day in F) the following effects were seen in females: a treatment-related decrease in body weight, adrenal weight associated with a reduction in individuals with haemorrhagic degeneration of the cortex, increased spleen weight with associated haemosiderin accumulation and pigment deposits in the Kupffer star cells in the liver. Based on these findings, a NOAEL of 300 ppm for females, equivalent to 23 mg/kg bw/d, was determined for systemic effects. Findings in this 2-year study were similar to those in repeat-dose toxicity studies conducted for 28- and 90-days. No carcinogenic effect was seen up to the top dose of 1000 ppm, equivalent to 55 mg/kg bw/d for males and 86 mg/kg bw/d for females respectively.

Mouse
The long-term toxicity and carcinogenic potential of tebuconazole was assessed in two studies conducted in the mouse over 21 months.

The first study tested doses of 20 – 180 ppm (6 – 81 mg/kg bw/d). Adverse and treatment-related effects on the liver, including fatty degeneration/vacuolation in both sexes, and an increase in bilirubin in females, were seen at a dose of 60 ppm and above (18 – 26 mg/kg bw/d). Increases in absolute and relative liver weights (statistically significant for males only), decreases in cholesterol in both sexes and reductions in erythrocyte counts in males were seen at the top dose of 180 ppm. Based on these findings, a NOAEL of 20 ppm for males and females, equivalent to 6 and 9 mg /kg bw/day in males and females respectively, was identified as no effects were seen at this dose level. No treatment-related tumour findings were observed up to the top dose of 180 ppm. (equivalent to 53 and 81 mg/kg bw/d for males and females respectively).

The second study tested higher doses of 500 and 1500 ppm (85 – 357 mg/kg bw/d) as the question of whether a maximum tolerated dose (MTD) had been reached in the first study was raised. Severe liver effects, which included enlargement of the liver, increased liver weights, single cell and focal necrosis, inflammation, bile duct hyperplasia and steatosis, accompanied by clinical-chemistry and haematological changes, were evident at both doses. Given the marked liver toxicity observed, particularly at the top dose of 1500 ppm, the RMS considers the MTD to have been exceeded in this study. Based on these findings, a LOAEL of 500 ppm equivalent to 85 and 103 mg /kg bw/d in males and females respectively, was identified for systemic effects. Liver tumours were significantly increased in both sexes at 1500 ppm (the top dose), a dose at which marked liver toxicity occurred. Increases in tumours were markedly above the range of spontaneous incidences observed in this mouse strain. A NOAEL for carcinogenicity in the mouse of 500 ppm, equivalent to 85 and 103 mg/kg bw/d for males and females respectively, was therefore identified.

Conclusion
The long-term toxicity and carcinogenic potential of tebuconazole has been investigated in a range of studies conducted in both the rat and the mouse. The mouse appeared to be the most sensitive species.

The lowest long-term systemic toxicity NOAEL, identified in the mouse, was 20 ppm (6/9 mg/kg bw/d in M/F), based on effects at 60 ppm (18/26 mg/kg bw/d in M/F). Tebuconazole was carcinogenic in the mouse, causing liver tumours in both sexes at 1500 ppm (280/357 mg/kg bw/d in M/F). A NOAEL for carcinogenicity of 500 ppm in males and females (85/103 mg/kg bw/d in M/F) was identified as no tumours were seen at this dose level.

Conclusion on classification for carcinogenicity in accordance with the CLP Regulation

The carcinogenicity of tebuconazole was investigated in cancer bioassays in rats (1 study) and mice (2 studies). Tebuconazole was not carcinogenic in rats but caused an increased incidence of liver adenomas and carcinomas in both sexes at the highest dose of 1500 ppm (280/357 mg/kg bw/d in M/F) in the second mouse study. A number of mechanistic studies (please see Vol 3, CA_B6 for further details) have shown that these liver tumours arise through activation of CAR/PXR receptors, with consequent altered gene expression, hypertrophy, and hepatocyte proliferation, leading to altered foci and eventually tumours. The available evidence has also shown that hepatocyte proliferation does not occur in human hepatocyte cultures exposed to tebuconazole, which clearly confirms that human hepatocytes are not sensitive to a key event in this MoA. On this basis, the RMS postulates that the resulting liver tumours are not relevant to humans. Classification of tebuconazole for carcinogenicity is therefore not warranted.

B.6.6. REPRODUCTIVE TOXICITY

The reproductive toxicity of tebuconazole has been investigated in numerous regulatory studies (a rat multi-generational study, developmental toxicity and developmental neurotoxicity studies in rats and developmental toxicity studies in rabbits and mice). There are also publications of relevance to reproductive toxicity from the open literature.

B.6.6.1. Generational studies

One oral multi-generational study (B.6.6.1.1/01) in the rat was described in the original DAR (2006). No new multi-generational studies have been submitted for the purposes of renewal.

B.6.6.1.1. *Two-generation dietary study in rats*

Previous evaluation	In DAR (2006) for original approval

Study ID	B.6.6.1.1/01
Study title	HWG 1608 – Two-Generation Study in Rats
Study Matrix ID	17
Test substance	(HWG 1608) Tebuconazole
Purity (%) **Batch no.**	95.2 Mixed batch with Fl. No. 132
Test animals	Male and female Wistar rats of the strain Bor:WISW (SPF Cpb)
Groups	25 males and 25 females/dose
Dose	0, 100, 300, or 1000 ppm in the food
Route	Oral, dietary
Vehicle	Plain diet
GLP	Yes
Guideline	OECD Guideline 416 (1983) and US EPA Pesticide Assessment Guidelines, Subdivision F, 1983.
Deviation	The following deviations from the OECD-Guideline 416 (2001) occurred: - Oestrus cycle and sperm parameters not measured in P and F1 - Vaginal opening, preputial separation, anogenital distance not measured. - Weight of the following organs was not determined: uterus, epididymides, prostate, seminal vesicles with coagulating glands and their fluids, brain, pituitary, and thyroid. - The following organs were not subject to histopathological examination: cervix, coagulating gland.
UK-RMS Acceptable	Acceptable – These deviations do not compromise the validity of the study and the adequacy of the dataset as some of the missing investigations have been covered by the available RDT

	studies, DNT studies and studies from the open literature.
DK-RMS Acceptable with restrictions	Acceptable with restrictions. Many of the above mentioned deviations from the current OECD TG416 cannot be examined in repeated dose studies because they need to be measured in studies with developmental exposure. Studies from the open literature typically use a lower groups size than guideline studies and are not necessarily included in the overall assessment of reproductive toxicity.
UK- RMS NOAEL	Reproductive toxicity: 1000 ppm (72 – 111 mg/kg bw/d) Parental and offspring toxicity: 300 ppm (22 – 34 mg/kg bw/d)
DK-RMS NOAEL	Reproductive toxicity: 300 ppm (22 – 34 mg/kg bw/d) Parental and offspring toxicity: 300 ppm (22 – 34 mg/kg bw/d)
Effects at the LOAEL	Reproductive toxicity: adverse effects on pre- and postnatal offspring survival ~~no effects up to~~ the top dose of 1000 ppm (72 – 111 mg/kg bw/d). Parental and offspring toxicity: Decreased food consumption is not statistically significant (8-11% of control, no change in first generation females), slightly retarded weight gains for parents and decrease in bodyweight (less than 10% and not considered toxicologically relevant in parents). Reduced birth weight (less than 10%, some generations) & bw ~~gain~~ for pups during development (15-25% at some ages). Organ weight decrease (absolute liver & kidney weight, not relative) secondary to decreased body weights in F1B parentals and possible dystocia in one dam-at1000 ppm (72 – 111 mg/kg bw/d).

Methods

Tebuconazole was tested in a two generation study in Wistar rats. Groups of 25 males and females were fed diets containing 0, 100, 300 or 1000 ppm (95.2 % pure) tebuconazole for 120 days before mating. No other ration was fed to the test animals throughout the study. The animals were observed for clinical or behavioural symptoms at least once daily. Food and tap water were supplied *ad libitum*. Food consumption and body weights were recorded during the whole study. The F_0 generation mated twice to yield first the F_{1A} generation and then the F_{1B} generation (and those dams that had not been inseminated were mated an extra time with proven fertile males to test their fertility – they all produced pups). The F_{1B} generation was mated (siblings mating was avoided) twice to yield F_{2A} and F_{2B} generations. All fertility data were recorded at all matings. Litters were reduced to – as far as possible – 4 male and 4 female pups each after 4 days. Viability data, lactation indices etc. were calculated. Pups of the F_{1A} generation and the F_{2A} generation were killed after weaning and were examined for external malformations. After weaning of the F_{2B} generation all animals were sacrificed and subjected to full necropsy, incl. measuring femur bones, and recording of organ weights. Parent animals were sacrificed as soon as possible after second mating or nursing the pups, respectively (read above with respect to "infertile" females and surely fertile males of the F_0 generation, though).

Feeding *ad libitum* with diets containing 0, 100, 300, or 1000 ppm tebuconazole resulted in the following daily test substance intakes:

Table 6.6-1. Study design and doses

Test group		**1**	**2**	**3**	**4**
Concentration in diet	[ppm]	0	100	300	1000
Dose per animal [mg/kg bw/day]	F_0-males	0	7.12	21.60	72.27
	F_1-males	0	9.24	27.06	97.20
	F_0-females	0	9.07	27.77	94.81
	F_1-females	0	11.10	33.87	111.40

Results

Parental animals

Clinical signs and mortality

Doses up to 1000 ppm caused no changes in behaviour and appearance of any animals. The mortality rate was not affected by treatment; two control females and one female in the 1000 ppm group died or were moribund, however, this was not considered treatment related.

Food intake

First generation: Food consumption was non-significantly decreased (-10 % change compared to control) in males but not in females at 1000 ppm (+ 7 % change compared to control) (Table 6.6-2). No effect was observed at 100 and 300 ppm.
Second generation: Food intake was non-significantly decreased in males (-8 % change compared to control) and females (-11 % change compared to control) at 1000 ppm. No effect was observed at 100 and 300 ppm.

Table 6.6-2. Food intake

Parameter	Generation	Dose (ppm)					
		0	100	*(%)[a]*	300	*(%)[a]*	1000	*(%)[a]*
Food intake [g/animal/day] – Males								
	F_0	21	20	*(-5)*	20	*(-5)*	19	*(-10)*
	F_{1B}	24	24	*(±0)*	24	*(±0)*	22	*(-8)*
Food intake [g/animal/day] – Females								
	F_0	15	16	*(+7)*	16	*(+7)*	16	*(+7)*
	F_{1B}	19	18	*(-5)*	19	*(±0)*	17	*(-11)*

[a]: % compared to control

Body weight
First and second generation:
Pre-mating: Statistically significantly and adverse (>10% change compared to control) lower body weights in males and females were observed in the 1000 ppm dose group (Table 6.6-3) at some time-points and in some generations. Body weights of the 100 and 300 ppm groups were unaffected.

Gestation and Lactation: Statistically significantly lower body weights in dams of the 1000 ppm dose group were observed, body weights decreased at all time points, however < 10 % change compared to control was seen at most timepoints and generations. Body weights of the 100 and 300 ppm groups were unaffected.

Table 6.6-3. Body weight

Parameter	Generation	Dose (ppm)					
		0	100	*(%)[a]*	300	*(%)[a]*	1000	*(%)[a]*
Body weight [g] – Males								
Week 0	F_0 1st pre-mating	92	92	*(±0)*	93	*(+1)*	91	*(-1)*
Week 17		351	343	*(-2)*	348	*(-1)*	327**	*(-7)*
Week 5	F_{1B} 1st pre-mating	97	92	*(-5)*	99	*(+2)*	82**	*(-15)*
Week 14		317	317	*(±0)*	321	*(+1)*	286**	*(-10)*
Body weight [g] – Females – F_0 generation								
Week 0	F_0 1st pre-mating	88	89	*(+1)*	90	*(+2)*	89	*(+1)*
Week 17		206	208	*(+1)*	206	*(±0)*	196*	*(-5)*
Gestation day 1	F_0 1st gestation	201	205	*(+2)*	200	*(±0)*	193	*(-4)*
Gestation day 20		284	282	*(-1)*	288	*(+1)*	263*	*(-7)*
Lactation week 22	F_0 1st lactation	241	241	*(±0)*	240	*(±0)*	223	*(-7)*
Lactation week 25		241	235	*(-2)*	240	*(±0)*	223**	*(-7)*
Gestation day 1	F_0 2nd gestation	218	224	*(+3)*	220	*(+1)*	204*	*(-6)*
Gestation day 20		311	293	*(-6)*	286	*(-8)*	284*	*(-9)*
Lactation week 34	F0 2nd lactation	263	255	*(-3)*	249	*(-5)*	233**	*(-11)*
Lactation week 37		246	247	*(±0)*	240	*(-2)*	229**	*(-7)*
Body weight [g] – Females – F_{1B} generation								
Week 5	F_{1B} 1st pre-mating	86	83	*(-3)*	89	*(+3)*	75**	*(-13)*
Week 14		192	192	*(±0)*	195	*(+2)*	180**	*(-6)*
Gestation day 1	F_{1B} 1st gestation	192	196	*(+2)*	195	*(+2)*	184	*(-4)*
Gestation day 20		278	286	*(+3)*	290	*(+4)*	265	*(-5)*
Lactation week 19	F_{1B} 1st lactation	245	245	*(±0)*	252	*(+3)*	221	*(-10)*
Lactation week 22		240	242	*(+1)*	244	*(+2)*	214**	*(-11)*
Gestation day 1	F_{1B} 2nd gestation	219	220	*(±0)*	221	*(+1)*	205**	*(-6)*

Parameter	Generation	Dose (ppm)					
		0	100	*(%)[a]*	300	*(%)[a]*	1000 *(%)[a]*
Gestation day 20		298	302	*(+1)*	314	*(+5)*	278* *(-7)*
Lactation week 32	F_{1B} 2nd lactation	247	242	*(-2)*	244	*(-1)*	225** *(-9)*
Lactation week 35		237	242	*(+2)*	242	*(+2)*	224 *(-5)*

[a] % compared to control
* statistically significant difference from control p≤0.05
** statistically significant difference from control p≤0.01

Reproductive toxicity
Mating behaviour, gestation indices and duration were comparable to controls after first and second mating of both generations (Tables 6.6-4 and 6.6-5). Fertility indices were not affected. Fertility indices at 100 ppm (both matings) and 300 ppm (only F_{1B}) were below the control figures but this is by the UK-RMS not regarded as an indication of reduced fertility since all 300 ppm females proved to be fertile when re-mated and at 1000 ppm, the fertility was comparable with the control values (Tables 6.6-4 and 6.6-5). Litter size was (statistically significantly) reduced at 1000 ppm in the F_0 generation, second mating only; however this effect was not repeated in the F_{1B} generation (both matings). Therefore, this was by the UK-RMS not considered treatment-related. The DK-RMS disagrees and consider it plausible that the reduced litter size could be an indication of increased postimplantation loss. Additionally, death of one dam (F0) in the 1000 ppm group was possibly related to dystocia. This dam was found moribund and sacrificed with uterus horns found to be very thick, beige coloured and hard.

Table 6.6-4. Reproductive performance – F_0 generation

Parameter		Dose (ppm)			
		0	100	300	1000
F_0 generation – first mating					
Mating index		100	96.0	100	96.0
Fertility index		88.0	75.0	88.0	87.5
Duration of pregnancy	mean	22.3	22.2	22.3	22.3
Gestation index		100	100	100	90.5
Litter size	mean	9.0	10.3*	9.5	7.6
Pup weight [g]	mean	6.0	5.7*	5.8	**5.6***
Sex ratio	male/female	97/107	95/91	105/120	90/87
Viability index		98.5	90.3**	95.2	**88.1****
Lactation index		95.2	88.9	91.1	**86.3***
Number of pups	% of control	100	91.2	110.3	86.8
F_0 generation – second mating					
Mating index		100	92.0	96.0	92.0
Fertility index		95.7	78.3	75.0	95.7
Duration of pregnancy	mean	22.0	21.9	22.2	22.2
Gestation index		100	94.4	100	95.5
Litter size	mean	9.1	8.4	7.7	**6.7***
Pup weight [g]	mean	5.7	5.6	5.7	5.6
Sex ratio	male/female	106/104	78/81	71/80	81/79
Viability index		94.5	89.4	94.2	88.5
Lactation index		92.1	76.1**	98.2*	**80.3***
Number of pups	% of control	100	75.7	71.9	76.2

* statistically significant difference from control p≤0.05
** statistically significant difference from control p≤0.01

Table 6.6-5. Reproductive performance – F_{1B} generation

Parameter	Dose (ppm)			
	0	100	300	1000
F_{1B} generation – first mating				
Mating index	100	100	96.0	100
Fertility index	96.0	96.0	95.8	92.0

Parameter		Dose (ppm)			
		0	**100**	**300**	**1000**
Duration of pregnancy	mean	22.3	22.0	22.1	22.0
Gestation index		100	100	100	100
Litter size	mean	10.5	11.2	11.2	10.2
Pup weight [g]	mean	5.9	5.5**	5.7	**5.3****
Sex ratio	male/female	123/132	123/148	129/132	116/123
Viability index		98.0	99.6	97.7	96.6
Lactation index		98.4	99.5	99.5	97.2
Number of pups	% of control	100	106.3	102.4	92.7
F_{1B} generation – second mating					
Mating index		100	100	100	100
Fertility index		95.8	84.0	92.0	84.0
Duration of pregnancy	mean	22.1	21.7	21.7	22.1
Gestation index		91.3	100	100	100
Litter size	mean	9.0	11.8**	11.4*	9.7
Pup weight [g]	mean	5.7	5.7	5.7	**5.3***
Sex ratio	male/female	97/117	110/143	136/137	108/105
Viability index		91.7	96.0	98.9**	91.1
Lactation index		97.3	97.0	97.2	97.9
Number of pups	% of control	100	118.2	127.6	99.5

* Significantly different from study controls ($p \leq 0.05$)

** Significantly different from study controls ($p \leq 0.01$)

F1B Parental post mortem results

Organ weights: Adult terminal body weights were significantly decreased in males and females of the 1000 ppm group; however this was seen at < 10 % change compared to control, therefore it was not considered toxicologically relevant (Table 6.6-6.). Statistically significantly decreased organ weights were observed in the 1000 ppm group in males and females. A ≥10 % change compared to control was only seen for absolute liver weight in males (however this was < 15 % change compared to control) and kidney weight in males. Therefore changes in organ weights were considered to be indirect effects related to the decreased body weight noted at this dose level and not treatment-related or adverse. Statistically significant deviations noted in the 100 and 300 ppm groups did not show dose correlation (Table 6.6-6.).

Table 6.6-6. Organ weights (F_{1B} generation)

Parameter		Dose (ppm)							
		0	**100**	*(%)[a]*	**300**	*(%)[a]*	**1000**	*(%)[a]*
Males – F_{1B} generation								
Body weight [g]		390	391	*(±0)*	390	*(±0)*	356**	*(-9)*
Liver [mg]	absolute	13694	13898	*(+1)*	12613	*(-8)*	**12071****	***(-12)***
	relative	3512	3546	*(+1)*	3230**	*(-8)*	3383	*(-4)*
Kidneys [mg]	absolute	2391	2360	*(-1)*	2408	*(+1)*	**2144****	***(-10)***
	relative	613	603*	*(-2)*	617	*(+1)*	604	*(-1)*
Adrenals [mg]	absolute	44	42	*(-5)*	40	*(-9)*	39	*(-11)*
	relative	11	11	*(±0)*	10	*(-9)*	11	*(±0)*
Females – F_{1B} generation								
Body weight [g]		237	238	*(±0)*	241	*(+2)*	224*	*(-5)*
Liver [mg]	absolute	9402	9485	*(+1)*	9923	*(+6)*	9196	*(-2)*
	relative	3960	3988	*(+1)*	4110	*(+4)*	4110	*(+4)*
Kidneys [mg]	absolute	1576	1554	*(-1)*	1612	*(+2)*	1458*	*(-7)*
	relative	666	652	*(-2)*	667	*(±0)*	651	*(-2)*
Adrenals [mg]	absolute	59	60	*(+2)*	63*	*(+7)*	57	*(-3)*
	relative	25	25	*(±0)*	26	*(+4)*	26	*(+4)*

[a] % compared to control

* Significantly different from study controls ($p \leq 0.05$)

Parameter	Dose (ppm)			
	0	**100** *(%)[a]*	**300** *(%)[a]*	**1000** *(%)[a]*

** Significantly different from study controls ($p \leq 0.01$)

Bone examination: Bone growth was not affected at any dose group.
Pathology: There were no treatment related effects observed in any dose group.

Offspring/developmental toxicity
Viability and clinical signs
No treatment-related clinical signs in either generation occurred. None of the pups showed grossly apparent malformations at birth or during lactation.

In both matings of the F_0 generation viability and/or lactation indices were slightly reduced compared to the concurrent control at 1000 ppm (lactation indices within historical control data) (Table 6.6-4.). No dose related effect was observed at 100 and 300 ppm. In both matings of the F_{1B} generation neither viability nor lactation indices were altered (Table 6.6-5.). Since the F_1 generation was much longer exposed to the compound the UK-RMS concludes that the reduction in the F_0 generation was most likely due to normal biological variability. The DK-RMS does not agree with this conclusion. The slight increase of stillborn pups at 300 and 1000 ppm (Table 6.6-7.) did not differ significantly from control values, did not show dose correlation, was in accordance with historical control data, and nearly all females with any stillbirths delivered dead pups only in one of the two litters. It is noted that the only historical control data found by DK RMS was for the lactation index and did not fulfill the five-year period, centred as closely as possible on the date of the index study. In group 0 and 1000 ppm group only one F_0 and F_{1B} female each delivered dead pups in both litters. This observation is considered by the UK-RMS to be within the normal variation expected for this endpoint in this rodent strain. DK-RMS does not agree with this conclusion and finds this to be an exposure-related adverse effect of perinatal exposure to tebuconazole, as similar effects have been observed in the majority of the other developmental toxicity studies (see overview Table 6.6-94and B.6.6.4 Overall summary on reproductive toxicity).

Table 6.6-7. Number pups at birth and ratio of males to females

Dose (ppm)	Number at birth					
	Total	Stillbirths (% out of total)	Male		Female	
			n	%	n	%
F_{1A} generation						
0	204	5 (2.4%)	97	48	107	52
100	186	0	95	51	91	49
300	225	16 (7.1%)	105	47	120	53
1000	177	17 (9.6%)	90	51	87	49
F_{1B} generation						
0	210	9 (4.2%)	106	50	104	50
100	159	8 (5%)	78	49	81	51
300	151	12 (7.9%)	71	47	80	53
1000	160	12 (7.5%)	81	51	79	49

Body weight
First and the second generation: The pup weights of the 100 and 300 ppm dose groups did not significantly differ in comparison to the respective control group weights (Table 6.6-8). Pup body weights and body weight gains in male and female pups were significantly lower throughout lactation in the 1000 ppm group (> 10 % change compared to control, and at some ages between 15-25% reductions were observed). The effects on pup body weight were by the UK-RMS considered to be secondary to maternal effects. The DK-RMS disagrees with this conclusion and does not find the markedly reduced pup body weights to be fully explained by the slight maternal toxicity which was seen in this dose group (body weight decreases of < 10% compared to controls, and no statistically significant changes in food intake in F0 or F1B dams).

Table 6.6-8. Pup body weight

Parameter	Generation	Dose (ppm)						
		0	100	*(%)[a]*	300	*(%)[a]*	1000	*(%)[a]*
Mean pup weight [g] – males & females combined								
LD 0 birth	F_{1A} generation	6.0	5.7*	*(-5)*	5.8	*(-3)*	**5.6***	*(-7)*
LD 4 prior reduction		10.2	9.4	*(-8)*	9.8	*(-4)*	**9.0****	*(-12)*
LD 4 after reduction		10.4	9.6	*(-8)*	10.0	*(-4)*	**9.0****	*(-13)*
LD 7 (week 1)		126	12.4	*(-2)*	13.2	*(+5)*	**10.7****	*(-15)*
LD 14 (week 2)		23.7	24.4	*(+3)*	24.3	*(+3)*	**20.0****	*(-16)*
LD 21 (week 3)		34.9	34.2	*(-2)*	37.0	*(+6)*	**30.3****	*(-13)*
LD 28 (week 4)		54.1	53.7	*(-1)*	56.6	*(+5)*	**47.8****	*(-12)*
LD 0 birth	F_{1B} generation	5.7	5.6	*(-2)*	5.7	*(±0)*	5.6	*(-2)*
LD 4 prior reduction		9.3	8.5	*(-9)*	10.2	*(+10)*	8.9	*(-4)*
LD 4 after reduction		9.4	8.5	*(-10)*	10.3	*(+10)*	8.8	*(-6)*
LD 7 (week 1)		12.7	11.2*	*(-12)*	13.6	*(+7)*	**11.4***	*(-10)*
LD 14 (week 2)		24.3	24.2	*(±0)*	25.6	*(+5)*	22.8	*(-6)*
LD 21 (week 3)		37.6	38.2	*(+2)*	38.7	*(+3)*	34.3	*(-9)*
LD 28 (week 4)		58.7	58.6	*(±0)*	60.2	*(+3)*	52.4	*(-11)*
LD 0 birth	F_{2A} generation	5.9	5.5**	*(-7)*	5.7	*(-3)*	**5.3****	*(-10)*
LD 4 prior reduction		9.9	9.3	*(-6)*	9.6	*(-3)*	**8.1****	*(-18)*
LD 4 after reduction		10.1	9.4*	*(-7)*	9.7	*(-4)*	**8.1****	*(-20)*
LD 7 (week 1)		12.7	12.1	*(-5)*	12.6	*(-1)*	**10.3****	*(-19)*
LD 14 (week 2)		23.4	22.7	*(-3)*	22.8	*(-3)*	**18.1****	*(-23)*
LD 21 (week 3)		36.5	35.6	*(-2)*	35.7	*(-2)*	**27.4****	*(-25)*
LD 28 (week 4)		56.0	56.4	*(+1)*	56.4	*(+1)*	**43.7****	*(-22)*
LD 0 birth	F_{2B} generation	5.7	5.7	*(±0)*	5.7	*(±0)*	**5.3***	*(-7)*
LD 4 prior reduction		9.2	9.1	*(-1)*	9.4	*(+2)*	**8.4****	*(-9)*
LD 4 after reduction		9.3	9.3	*(±0)*	9.7	*(+4)*	**8.5****	*(-9)*
LD 7 (week 1)		12.6	12.0	*(-5)*	12.6	*(±0)*	**10.0****	*(-21)*
LD 14 (week 2)		24.0	24.0	*(±0)*	24.3	*(+1)*	**19.7****	*(-18)*
LD 21 (week 3)		36.0	36.8	*(+2)*	38.1	*(+6)*	**30.5****	*(-15)*

Key
LD lactation day
(%)[a] % compared to control
* Significantly different from study controls ($p \leq 0.05$)
** Significantly different from study controls ($p \leq 0.01$)

UK-RMS Conclusion

There were no effects on reproduction up to the top dose. Therefore, a NOAEL of 1000 ppm (72.3 – 97.2 mg/kg bw/day in males and 94.8 – 111.4 mg/kg bw/day in females) can be identified for reproductive toxicity. This is a change from the previously agreed NOAEL for reproductive toxicity of 300 ppm (21.6 – 27.1 mg/kg bw in males and 27.8 – 33.9 mg/kg bw in females) based on reduced litter size in one mating only in the F_0 generation only. The UK RMS considers this effect to be not treatment-related.

In adult parental animals, decreases in food consumption, retarded body weight gains and reduced organ weights were also seen at the top dose of 1000 ppm; a NOAEL for parental toxicity of 300 ppm (21.6 – 27.1 mg/kg bw in males and 27.8 – 33.9 mg/kg bw in females) was therefore identified.

In offspring, there were reduced body weight gains at the top dose of 1000 ppm; no adverse effects were noted at any other dose. A NOAEL for offspring toxicity at the next dose of 300 ppm (21.6 – 27.1 mg/kg bw in males and 27.8 – 33.9 mg/kg bw in females) was therefore identified.

The parental and offspring NOAELs are the same as those agreed during the first review of tebuconazole.

B.6.6.1.1 Discussion and conclusion by DK-RMS:	DK-RMS notes that adverse reproductive toxicity effects were clearly seen at the oral dose of 1000 ppm (top dose). In mating of the F0 generation (in cohort a&b) a higher number of stillborn pups was observed, statistically significant lower viability index (i.e. pup survival from birth to PND 4) and lower lactation index (i.e. pup survival from PND4-21), and a statistically significant lower litter size which could be an indication of increased postimplantation loss (table 6.6-4). Some of these effects were also sporadically found in the lower dose groups (100 and 300 ppm), but here the patterns were not consistent between the two cohorts (a&b) and often did not show clear dose-response relationships, and were therefore by the DK-RMS considered likely to be chance findings. HCD live birth index and littersize RMS-DK requested detailed information of the supplied HCD used in the discussion of results in the 2-generation study by applicant. **"RMS Question:** If we are to review the HCD again, please submit the HCD including relevant minimum details/relevant period as specified above for pup weight at birth, lactation index and viability index. For example in an excel file. **BCS Answer:** We have attached the Historical control data for two specific end points; Litter size at birth and number of stillborn pups. The historical control data are provided in an Excel format and includes separate data tabs for individual studies which details individual litter responses within control groups. It was considered that the data presented represents the key analysis needed to demonstrate a lack of significant effect between the treated group and the concurrent control particularly in terms of offspring viability. The purpose of the Historical control data is to show that the values presented for the study concurrent control group are representative of the normal responses for the strain of rat used. This then allows a realistic assessment of responses between concurrent control and treated groups for the study itself. The historical control data are being used to assess the key factors associated with litter viability for each of the generations and matings for the two generation reproduction study with tebuconazole It is acknowledged that some data may be considered to be outside of the period September 1982 to September 1987. A question may be raised as to how this 5 year period is interpreted. All the studies listed in the "summary" tab of the excel file did have an overlap of some part of the in life phase of the study with this 5 year period but may not necessarily have started the in life phase during this 5 year period. Due to a potential difference of interpretation of what constitutes a 5 year period for data inclusion, a separate data tab("excluded") is included in the excel file where the summary of the data excludes those studies that may be considered outside of the allowable Historical Control Data. A third tab labelled "comparison" has also been included which shows the results of inclusion/exclusion of certain studies. It is our interpretation that there are no remarkable differences seen when data are included/excluded. Therefore in order to support the robustness of the data set by inclusion of all studies this lack of significant differences in the include/exclude data set provides evidence of the consistency of response for this particular strain of rat and that the values for the concurrent control group are within the acceptable range." **RMS:** Applicant supplied data on litter size at birth and number of stillborn/number of litters. These data was stated to be from the performing laboratory (Bayer) and the same rat strain and breeder as used in B.6.6.1.1/01. The live birth index was used instead of number of stillborn/litters used for comparison because the total number of pups is then taken into account. This information was also supplied in the HCD reports from the performing

laboratory received by DK-RMS. Data from studies performed 2.5 years on either side of the B.6.6.1.1/01 study was included in the ranges.
B.6.6.1.1/01:

Dosis/ dose	Anzahl bei Geburt/number at birth				%	
ppm	total	tot/ dead	♂	♀	♂	♀
F1A-Generation/F1A generation						
0	204	5	97	107	48	52
100	186	0	95	91	51	49
300	225	16	105	120	47	53
1000	177	17	90	87	51	49
F1B-Generation/F1B generation						
0	210	9	106	104	50	50
100	159	8	78	81	49	51
300	151	12	71	80	47	53
1000	160	12	81	79	51	49

Dosis/ dose	Anzahl bei Geburt/number at birth				%	
ppm	total	tot/ dead	♂	♀	♂	♀
F2A-Generation/F2A generation						
0	255	3	123	132	48	52
100	271	3	123	148	45	55
300	261	3	129	132	49	51
1000	239	4	116	123	49	51
F2B-Generation/F2B generation						
0	214	8	97	117	45	55
100	253	5	110	143	43	57
300	273	11	136	137	50	50
1000	213	10	108	105	51	49

	B.6.6.1.1/01 study:
Dose (ppm)	live birth index (%)=number viable pubs*100/number of pups
	F1A
0	97,5
100	100,0
300	92,9
1000	85,5
	F1B
0	95,7

100	95,0
300	92,1
1000	92,5
	B.6.6.1.1/01 studiet:
Dose (ppm)	live birth index (%)=number viable pubs*100/number of pups
	F2A
0	98,8
100	98,9
300	98,9
1000	98,3
	F2B
0	96,3
100	98,0
300	96,0
1000	95,3

HCD live birth index range recorded in the HCD:
Within 5 years,
Based on 12 studies (F1A) and 11 studies (F1B):
F1A: 96.6-100 %
F1B: 92.3-100 %
Based on 8 studies:
F2A: 97.7-100 %
F2B: 93-100 %
In the 2 generation study, the live birth index was decreased below the HCD range at 1000 mg/kg bw/day in the mating of F0. This was most apparent in F1A.

Mean Litter size at birth ranges recorded in the HCD:
Based on 11 studies (F1A) and 10 studies (F1B):
F1A: 9.05-11.5
F1B: 7.9-10.6
Based on 8 (F2A) and 7 (F2B) studies:
F2A: 9.4-11.7
F2B: 8.96-11.3

Mean litter size at high dose (1000 mg/kg bw/day) in the B.6.6.1.1/01 study:
F1A: 7.6
F1B: 6.7
F2A: 10.2
F2B: 9.7

Mean litter size in B.6.6.1.1/01was below the HCD range for F1A and F1B at 1000 mg/kg bw/day thus the HCD do not change the conclusion.

The adverse effects of tebuconazol on offspring survival (both pre-and postnatally) were not seen in the F2 generation. However, in both the F1 and F2 generations statistically significant lower offspring body weights were consistently seen throughout the lactation period in the high dose group (males and females combined). These body weight reductions were seen in all offspring cohorts, but the effects were most marked in the 2nd generation (both cohort

	a&b), where offspring body weights from lactation day 7-21 were 15-22% lower in the high dose group, compared to controls. In summary the study showed that in the F1 generation a dose of 1000 ppm resulted in markedly increased offspring mortality and moderately reduced offspring growth, whereas in the F2 generation there was no increase in offspring mortality but even more marked reductions in postnatal offspring growth. Generally the body weight reductions seen in the offspring were more marked than the corresponding reductions on maternal weight during the lactation period (5-11% decrease), indicating that tebuconazole caused specific developmental toxicity effects in the offspring. The DK-RMS finds it unlikely that all of the adverse effects observed in the offspring were unspecific consequences, secondary to maternal toxicity. This is further supported as similar adverse reproductive effects (including lower offspring body weights and increased offspring mortality) are consistently seen in other oral developmental toxicity studies in rats, as well as in studies in mice and rabbits, further highlighting the relevance of the effects observed in the present study (see section B.6.6.4 Overall summary on developmental toxicity by DK-RMS). The DK-RMS concludes that the NOAEL for reproductive toxicity in this study was 300 ppm, and not 1000 ppm as suggested by the UK-RMS. This is consistent with the previously agreed NOAEL for reproductive toxicity at the first peer review of the substance and also consistent with the reproductive NOAEL provided in the study report provided by applicant for this 2-generation study.

B.6.6.2. Developmental toxicity studies

B.6.6.2.1. *Rats*

Two oral developmental toxicity studies in the rat were described in the original DAR (2006) (B.6.6.2.1.1/01 and B.6.6.2.1.1/02) and are reproduced below. In addition a new study to investigate maternal toxicity in pregnant rats after oral administration of tebuconazole was submitted for the purpose of renewal (B.6.6.2.1.1/03); this study served as an investigative study only and was not used to identify NOAELs.

Two oral developmental neurotoxicity studies in the rat were described in the original DAR (2006) (B.6.6.2.1.2/01
and B.6.6.2.1.2/01). In addition, a review of these developmental neurotoxicity studies was described in the original DAR (2006) (B.6.6.2.1.2/03).

Two dermal developmental toxicity studies in the rat were described in the original DAR (2006) (B.6.6.2.1.3/01 and B.6.6.2.1.3/02) and are included below.

B.6.6.2.1.1. Developmental toxicity in rats after oral exposure

a)

Previous evaluation	In DAR (2006) for original approval

Study ID	B.6.6.2.1.1/01
Study title	HWG 1608 (Proposed common name ethyltrianol). Study for Embryotoxic Effects on Rats after Oral Administration
Matrix ID	18
Study dates	March to April 1984
Test substance	(HWG 1608) Tebuconazole
Purity (%) **Batch no.**	93.4 16007/83
Test animals	Mated (until sperm positive vaginal smear) female WISW rats
Groups	25/dose
Dose	0, 10, 30, 100 mg/kg bw/day on days 6-15 post mating. Male rats were used only for mating and remained untreated.
Route	Oral by gavage

Vehicle	0.5% Cremophor solution. Total volume applied 10 mL/kg bw
GLP	Yes
Guideline	No. Method used is comparable to OECD guideline 414 (1981) "Teratogenicity" in older versions (changed in 2001)
Deviation	The following deviations from the OECD-Guideline 414 (2001) occurred: - Food consumption was not recorded. - Dosing was performed during the period of organogenesis. - Caesarean section was performed one day too early resulting in very small foetuses. - Less than 50% of the foetuses were examined for visceral alterations. - Reporting is not sufficient – no raw data presentation. No corpora lutea data were given. No measurement of crown-rump length. - Numbers of pre- and post-implantation losses were not given in the report but were re-evaluated based on raw data. The deviations are not found to totally compromise the results with respect to embryo- or developmental toxicity.
Acceptable	Acceptable - with restrictions.
NOAEL	Maternal toxicity: 10 mg/kg bw/day Developmental toxicity: 30 mg/kg bw/day
Effects at the LOAEL	Maternal toxicity: reduced body weight gain at 30 mg/kg bw/day during the treatment. Developmental toxicity: an increased number of external malformations, a higher incidence of post-implantation losses and decreased foetal body weight at 100 mg/kg bw/day.

Methods

Groups of 25 inseminated Wistar rats were given by stomach tube on day 6 to day 15 of gestation daily doses of 0, 10, 30 or 100 mg/kg bw tebuconazole (93.4% pure). The test substance was suspended in 0.5% Cremophor solution and was administered at 10 mL/kg bw (0, 0.1, 0.3 or 1 %, respectively).

The dams were examined daily for clinical signs, appearance and behaviour and weights were recorded regularly (intervals not stated). Foetuses were delivered by Caesarean section on day 20 of gestation and were weighed, sex determined, and examined for either visceral malformations (Wilson staining) or skeletal deviations and malformations (Dawson staining). The uteri were examined for number of resorptions and placental weights.

Results

Maternal toxicity

Doses of 30 mg/kg bw/day and above resulted in maternal toxicity (Table 6.6-9). The dose of 30 mg/kg bw/day revealed a slightly decreased body weight gain during the first treatment days, which was not compensated, so that the overall weight gain during the treatment period was also decreased (by 16 % change compared to control). The dose of 100 mg/kg bw/day revealed a distinct body weight loss after start of treatment as well as a distinctly decreased body weight gain (by 74 % compared to control) during the treatment period. Furthermore, faecal alterations occurred at 100 mg/kg bw/day.

Table 6.6-9. Maternal effects

Parameter	**Control data**	**Low dose 10 mg/kg** *(%)[a]*	**Medium dose 30 mg/kg** *(%)[a]*	**High dose 100 mg/kg** *(%)[a]*
Number of dams examined [*n*]	22	18	21	24
Clinical findings[1)] [*n*]	0	0	0	0
Mortality of dams [*n*] [%]	0	0	0	0
Abortions [*n*]	0	0	0	0
Body weight gain: [g] during pregnancy	74.0	76.1 (+3)	79.1 (+7)	**61.0*** **(-18)**
during treatment	23.5	21.5 (-9)	**19.7*** **(-16)**	**6.2**** **(-74)**

Parameter	Control data	Low dose 10 mg/kg	Medium dose 30 mg/kg	High dose 100 mg/kg
Pregnancies [%]	88.0	76.0	84.0	100.0
Necropsy findings in dams dead before end of test [*n*]	0	0	0	0

1) During application of test substance.
* Significantly different from study controls ($p \leq 0.05$)
** Significantly different from study controls ($p \leq 0.01$)
(%)[a] % compared to control

Developmental toxicity
The dose of 30 mg/kg bw/day was tolerated without effects on intrauterine development (Table 6.6-10.). 100 mg/kg bw/day resulted in an increased resorption rate. There was also decreased foetal weight as shown by the higher number of small foetuses (< 3 g described as "runts") as well as an increased incidence of external malformations (Table 6.6-11 and 6.6-12.).

Table 6.6-10. Intrauterine development

Parameter	Control data		Low dose 10 mg/kg	Medium dose 30 mg/kg	High dose 100 mg/kg
	Historical[1)]	Study			
Corpora lutea					
Implantations (/dam)	-	10.2	10.7	11.0	10.9
Resorptions (/dam)	0.6 – 2.3	2.3	2.7	1.4	3.7[2)]
early	no HCD	0.9	1.5	1.0	1.4
late	no HCD	1.4	1.2	0.4	2.3
Total number of foetuses	-	173	152	201	174
Total number of litters	-	22	18	21	24
Foetuses / litter	-	7.9	8.0	9.6	7.0
Dead foetuses / litter	-	0	0	0	0
Foetus weight (mean) [g]	-	3.48	3.50	3.38	3.11**
Placenta weight (mean) [g]	-	0.65	0.62	0.64	0.64
Foetal sex ratio (m/f)	-	4.2/3.7	4.1/3.7	5.3/4.3	3.8/3.2
Small foetuses < 3 g F	no HCD	6.94	6.58	10.45	36.21
L	no HCD	40.91	38.89	58.14	79.17

* Significantly different from study controls ($p \leq 0.05$)
** Significantly different from study controls ($p \leq 0.01$)
F: Foetal incidence (%)
L: Litter incidence (%)
1): Historical control data range from 1983 – 1988 (BCS studies) – however no information on lab and strain.
2): 1 Female (No. 9575) with total resorptions (10) not included for calculations

Table 6.6-11. Examination of the foetuses

Parameter	Study control	Low dose 10 mg/kg	Medium dose 30 mg/kg	High dose 100 mg/kg
External malformations*1 [%]	1.7	0.7	2.0	**6.9***
Skeletal variants [%]	26.6	25.7	24.4	23.0

Parameter	Study control	Low dose 10 mg/kg	Medium dose 30 mg/kg	High dose 100 mg/kg

*1: Control group malformations: kink in spinal column, hydrocephalus internus, and microphthalmia – one sided (3 foetuses affected)
10 mg/kg bw group malformation: microphthalmia – one sided (one foetus affected)
30 mg/kg bw group malformations: microphthalmia – one sided (2), anophthalmia - both sides, kink in spinal column (4 foetuses affected)
100 mg/kg bw dose group: microphthalmia – one sided (7), anophthalmia – one sided (4), dysplasia of scapula and long bones, exencephaly + spina bifida and other malformations, encephalomeningocele + macroglossia and other malformations (12 foetuses affected).
* Significantly different from study controls ($p \leq 0.05$)
** Significantly different from study controls ($p \leq 0.01$)

Table 6.6-12. Foetal findings

Parameter		Control data		Low dose 10 mg/kg	Medium dose 30 mg/kg	High dose 100 mg/kg
		Historical [1)]	Study			
External malformations						
Foetuses affected			3	1	4	12
Litters affected			3	1	3	9
Total	F	0.0 – 3.0	1.7	0.7	2.0	**6.9***
	L	0.0 – 30.0	13.6	5.6	14.3	**37.5**
Microphthalmia	F	0.0 – 1.95	0.6	0.7	1.0	**4.0**
	L	0.0 – 18.18	4.6	5.6	9.5	**25.0**
Anophthalmia	F	0.0 – 0.52	0.0	0.0	0.5	**0.6** [2)]
	L	0.0 – 5.26	0.0	0.0	4.76	**4.17**
Hydrocephalus	F		0.6	0.0	0.0	0.0
	L		4.5	0.0	0.0	0.0
Bent spine	F		0.6	0.0	0.5	0.0
	L		4.5	0.0	4.8	0.0
Multiple malformations	F		0.0	0.0	0.0	**2.3** [3)]
	L		0.0	0.0	0.0	**16.67**

F: Foetal incidence (%) L: Litter incidence (%)
*/** significantly different from study controls ($p \leq 0.05$ / $p \leq 0.01$)
[1)] Historical control data ranges from 1983 – 1988 (BCS studies) – (55 studies; 9482 fetuses, 960 litters; same rat strain and test laboratory as in the present study).
[2)] 1 foetus (+ 2 together with other malformations),
[3)] narrow orbit; exencephaly, spina bifida, S-shaped spinal column, macroglossia, etc.; dysplasia of scapula and long bones; encephalomeningocele, macroglossia, hydronephrosis

DK RMS Table with overview of results

Dose, mg/kg bw/d	Ref	Maternal bw gain in pregnancy (and other maternal effects if relevant)	Postimpl loss/fetal death	Litter size	Fetal weight/ pup birth weight	Weight of litter	Maternal bw gain vs litter weight	External malformations	Skeletal anomalies
10,	ID 18	-	-		-			-	
30,	ID 18	↓ in first treatment days resulting in 16% reduced gain during treatment period (bw gain 3.8 g	-	- C: 7.9, Exp: 9.6	- C: 3.48 g Exp: 3.38 g (Runts: slight ↑ incidence from 6.94 to 10.45 %)	(↑) C: 7.9*3.48 g = 27.5 g Exp: 9.6*3.38 g = 32.4 g.		-	

Dose, mg/kg bw/d	Ref	Maternal bw gain in pregnancy (and other maternal effects if relevant)	Postimpl loss/fetal death	Litter size	Fetal weight/ pup birth weight	Weight of litter	Maternal bw gain vs litter weight	External malformations	Skeletal anomalies
		lower than control during exposure). No change in bw gain during whole pregnancy (5.1 g *heavier* than control).				Litter weight is **4.9 g higher** in exposed than controls			
100,	ID 18	↓ by 74% (bw gain 17.3 g lower than in control group). 18% ↓ in bw gain during whole pregnancy (bw gain **13 g lower** than controls during whole pregnancy).	↑	↓ from 7.9 to 7.0.	↓ from 3.48 to 3.11 g. (Small fetuses/runts: incidence ↑ from 6.94 % to 36.21%)	C: 7.9*3.48 g = 27.5 g Exp: 7.0*3.11 g = 21.77 g. Litter weight is **5.73 g lower** in exposed than controls.	The reduced litter weight (5.7 g) **partly explains** the lower BW gain (13 g) in the dam.	↑ from 1.7 to 6.9 (microphthalmia – one sided (7), anophthalmia – one sided (4), dysplasia of scapula and long bones, exencephaly + spina bifida and other malformations, encephalomeningocele + macroglossia and other malformations (12 foetuses affected))	

Exp: exposure group, C: control group

UK RMS Conclusion

In this limited teratogenicity study (similar to OECD guideline no. 414) with oral (gavage) dosing of mated female Wistar rats on days 6 - 15 of gestation maternal toxic effects were recorded in groups administered 30 and 100 mg/kg bw/day as significantly reduced weight gains (-16 % and -74 % respective change compared to control) were seen. No adverse effects were recorded at 10 mg/kg bw/d, which was therefore identified as the NOAEL for maternal toxicity. Developmental toxicity (increased number of total external malformations and microphthalmia, a higher incidence of post-implantation losses/resorptions and decreased foetal body weight) was evident at the top dose of 100 mg/kg bw/day. There was no evidence of developmental toxicity in the low- and mid-dose groups. Therefore, the NOAEL for developmental toxicity was the mid-dose level of 30 mg/kg bw/day. These are the same NOAEL values agreed during the first review of tebuconazole. It is noted that the developmental effects occurred in the presence of significant maternal toxicity.

B.6.6.2.1.1a Discussion and conclusion by DK-RMS:	DK-RMS agrees with this conclusion, but notes that dam body weight gain in the 30 mg/kg bw/day group was only marginally affected during the dosing period, but not during pregnancy. The maternal effects observed at the dose of 30 mg/kg bw/day were therefore very mild and not necessary toxicologically relevant and that actual maternal body weight was most likely not significantly affected in this dose. It is likely that the lower maternal body weight gain in the high dose group was partially explained by lower fetal weight and smaller litter size. For effects on intrauterine development, according to applicant, lab and strain was not available for HCD. The relevance is therefore considered questionable and concurrent control should be used for comparison. Moreover, no HCD were included in the study report or could be found in the dossier for DK-RMS to check. HCD on fetal findings were not verified by DK-RMS, as they seem to not be included in the study report or in the dossier. However, the available HCD do not change the conclusion of the study.

b)

Previous evaluation	In DAR (2006) for original approval.

Study ID	B.6.6.2.1.1/02
Study title	Embryotoxicity Study (including Teratogenicity) with HWG 1608 Technical in the rat
Matrix ID	19
Test substance	(HWG 1608) Tebuconazole technical
Purity (%) **Batch no.**	98.3 16002/85
Test animals	Mated female Wistar/HAN rats
Groups	25/dose
Dose	0, 30, 60, 120 mg/kg bw/day administered on day 6-15 post mating
Route	Oral by gavage
Vehicle	Distilled water with 0.5% Cremophor EL. Application volume: 10 mL/kg bw/day
GLP	Yes
Guideline	OECD guideline 414 for the testing of chemicals – Teratogenicity – and EPA Pesticide Assessment Guidelines § 163.83-3 (Teratogenicity Study). Both the EPA and the OECD guidelines have been revised (OECD guideline 414 in 2001) with extension of the dosing period to cover more than the organogenesis period and a few minor changes.
Deviation	The following deviations from the OECD-Guideline 414 (2001) occurred: - Food consumption was recorded at six-day intervals instead of three-day intervals. - Dosing was performed during organogenesis (from day 6-15 post mating). The deviations are not found to totally compromise the results with respect to embryo- or developmental toxicity.
Acceptable	Acceptable
Amendment to the report	Additional historical control data (1985 – 1988)
NOAEL	Maternal toxicity: 30 mg/kg bw/day Developmental toxicity: 60 mg/kg bw/day
Effects at the LOAEL	Maternal toxicity: reduced body weight gain and feed intake and increased liver weights at the next highest dose (60 mg/kg bw/day). In the high dose group (120 mg/kg bw/day) the reduced litter weight fully explains the reduced maternal bw gain during pregnancy. Developmental toxicity: based on higher incidence of resorptions, reduced ossification, decreased foetal weight and an increased incidence of skeletal variations and anomalies at the top dose (120 mg/kg bw/day).

Methods

The study was performed in accordance with Swiss, EPA (US) and OECD principles of GLP. Four groups of each 25 mated female Wistar/HAN rats were given on day 6-15 post mating daily doses of tebuconazole Technical.

The substance was suspended in 0.5% Cremophor EL solution. The dosed volumes of 10 mL/kg bw were corrected each day according to the actual body weight of each animal. The animals were weighed each day of the study and food consumption was recorded on days 6, 11, 16 and 21 post mating. The animals were inspected for deaths and clinical signs at least twice daily.

The dams were killed by CO_2 asphyxiation on day 21 after mating and the uteri were removed by Caesarean section. The livers of all females were weighed and stored for possible processing and histological examination. The post mortem examination performed included gross macroscopic examination of all internal organs, with emphasis on the uterus, uterine contents, position of foetuses in the uterus and number of corpora lutea. All results were recorded. The foetuses were removed from the uterus, sexed, weighed individually, examined for gross external abnormalities and allocated to either Wilson's slicing technique for examination of viscera and brain or to a modified Dawson stain technique for examination of skeletal abnormalities. Descriptions of all abnormalities were recorded. The uteri (and contents) were weighed. Non pregnant uteri were placed in aqueous ammonium sulphide to accentuate possible haemorrhagic areas of implantation sites.

Results

Maternal toxicity

Feed intake and body weight gain were affected at 60 mg/kg bw/day and 120 mg/kg bw/day (body weight gain of -15 % and -29 % respectively compared to control during treatment) (Table 6.6-13.). Further target effects were evident (statistically significantly increased liver weight, however < 15 % change compared to control) at this dose. These effects were more pronounced at 120 mg/kg bw/day (15 % change compared to control for relative liver weight). The feed intake at this dose level was decreased by as much as 20 % (GD 6-11) when compared to the control group. Further, the females of this group revealed a marked body weight loss (-5 g) during the first treatment days and a distinctly decreased body weight gain (-60 %) from gestation day 6-11 when compared to the control group.

Of the necropsy findings, only black/brown coloured fluid in the uterus in 9/25 dams in the 120 mg/kg bw dose group was significantly different from controls.

Table 6.6-13. Maternal toxicity

Parameter	**Control data**		**Low dose 30 mg/kg**		**Medium dose 60 mg/kg**		**High dose 120 mg/kg**	
	Historical	**Study**		***(%)[a]***		***(%)[a]***		***(%)[a]***
Number of dams examined	505	24	24		22		24	
Clinical findings[1)]		0	0		0		0	
Mortality of dams (%)	0	0	0		0		0	
Pregnancies (%)	94.1	96.0	96.0		88.0		96.0	
Abortions	0	0	0		0		0	
Body weight gain (g) GD 0 - 6		19	20	*(+5)*	20	*(+5)*	20	*(+5)*
GD 6 – 9		8	7	*(-12)*	2	*(-75)*	-3	*(-138)*
GD 6 – 11		20	16	*(-20)*	13	*(-35)*	**8**	***(-60)***
GD 11 – 16		21	24	*(+14)*	22	*(+5)*	21	*(±0)*
GD 16 – 21		55	53	*(-4)*	60	*(+9)*	53	*(-4)*
During treatment GD 6 – 16		41	40	*(-2)*	**35**	***(-15)***	**29**	***(-29)***
Until test end GD 6 - 21		96	93	*(-3)*	95	*(-1)*	82*	*(-15)*
Food consumption GD 0 – 6		20.6	20.1	*(-2)*	20.5	*(±0)*	20.8	*(+1)*
GD 6 – 11		20.2	19.7	*(-2)*	18.4	*(-9)*	**16.2**	***(-20)***
GD 11 - 16		22.6	22.1	*(-2)*	21.4	*(-5)*	20.1	*(-19)*
GD 16 – 21		23.0	23.3	*(+1)*	24.2	*(+5)*	24.3	*(+6)*
During treatment GD 6 - 16		21.4	20.9	*(-2)*	**19.9***	***(-7)***	**18.2***	***(-15)***
Terminal body weight mean (g)		320	314	*(-2)*	322	*(+1)*	303*	*(-5)*
Liver weights absolute weight (g)		11.51	11.55	*(±0)*	**12.50***	***(+9)***	**12.49***	***(+9)***
relative to body weight (%)		3.59	3.68	*(+3)*	**3.89****	***(+8)***	**4.12****	***(+15)***
Necropsy findings in dams dead before end of test		0	0		0		0	

Parameter	Control data	Low dose 30 mg/kg	Medium dose 60 mg/kg	High dose 120 mg/kg

1): during application of test substance.
(%)[a]: compared to study control.
GD: gestation day.
* Significantly different from study controls *($p \leq 0.05$)*
** Significantly different from study controls *($p \leq 0.01$)*

Developmental toxicity
The dose of 60 mg/kg bw/day was tolerated without effects on intrauterine development (Table 6.6-14.). Skeletal examination revealed statistically significant increases in the incidence of a number of anomalies and variants in the 120 mg/kg bw dose group: super-numerary ribs, non-ossified cervical vertebrae nos. 1-6, non-ossified sacral vertebral arches nos. 6 and 7, reduced ossification of various phalangeal nuclei and incompletely ossified sternebra no. 2 (Table 6.6-15.). The top dose of 120 mg/kg bw/day resulted also in increased resorption rates (early and late) (Table 6.6-14.), retarded foetal development (decreased foetal weight, accumulation of fluid in the thoracic cavity, incomplete ossification of further skeletal elements), an increased incidence of dumbbell shaped or bipartite thoracic vertebrae as well as in a slightly increased incidence of foetuses with supernumerary ribs. The percentage of foetuses with supernumerary ribs (22 % left, 21 % right) lay within the normal variation range for the strain of rats used (up to 25 or 21 %, respectively) (Table 6.6-16.) and is thus considered only a marginal effect. Therefore, the dose of 120 mg/kg bw/day produced a number of developmental effects (embryo lethality, reduced ossification, decreased foetal weight and an increased incidence of skeletal anomalies). These effects occurred in the presence of significant maternal toxicity (Table 6.6-13, decreased body weight gain, reduced food consumption and increased liver weight).

Table 6.6-14. Intrauterine development

Parameter	Control data		Low dose 30 mg/kg	Medium dose 60 mg/kg	High dose 120 mg/kg
	Historical 1)	Study			
Corpora lutea / dam	12.3 – 14.9	14.9	14.5	14.3	15.4
Implantations / dam	11.0 – 13.6	12.6	12.2	12.6	12.4
Resorptions / dam	0.3 – 1.1	0.6	0.8	1.0	**2.8***
early	0.3 – 1.1	0.6	0.8	0.9	**1.9**
late	0.0 – 0.2	0.0	0.0	0.1	**0.9**
Pre-implantation loss [% corp. lutea]	2.3 – 15.4	15.4	16.1	11.8	19.5
Post-implantation loss [% implant.]	3.0 – 9.2	4.6	6.9	7.6	22.1*
Total number of foetuses	8822	288	271	256	232*
Total number of litters	763	24	24	22	24
Foetuses / litter	10.3 – 12.9	12.0	11.3	11.6	9.7*
Dead foetuses / litter	0	0	0	0	0
Foetal sex ratio (males/females)		139/149	128/143	130/126	117/115
Foetus weight mean (g)	4.5 – 4.9	4.7	4.7	4.6	**4.1***
Dams with uterus alterations 2)		0	0	0	9*

*/** significantly different from study controls ($p \leq 0.05$ / $p \leq 0.01$)
1) Historical control data range from 1985-1988 (31 studies), species rat, strain Wistar/HAN (same species and strain), conducted in the same lab (same main author).
2 uterus filled with black/brown fluid

Table 6.6-15. Examination of the foetuses

Parameter	Control data		Low dose 30 mg/kg	Medium dose 60 mg/kg	High dose 120 mg/kg
	Historical	Study			
External malformations*1 [%]	0.16	0	0	0	0.86
External anomalies [%]		0	0	0	0

Parameter	Control data		Low dose	Medium dose	High dose
	Historical	Study	30 mg/kg	60 mg/kg	120 mg/kg
Skeletal anomalies*2 [%]	2.25	1.39	1.46	1.57	**6.90**
Skeletal variants*3 [%]					
Visceral malformations*4 [%]	0.15	0	0	0	1.72
Visceral anomalies*5 [%]		0	0.75	0	3.45

*1: One foetus with agnathia, microstomia, and anophthalmia one foetus without tail.
*2: Lower thoracic vertebral centrum either dumbbell shaped or bipartite
*3: At the 120 mg/kg bw dose group, the incidence of supernummary ribs, non-ossified cervical vertebrae nos. 1-6, sacral vertebral arches nos. 6 and 7 and various phalangeal nuclei and incompletely ossified sternebra no. 2 were increased and significantly different from controls.
*4: Same as *1
*5: Four foetuses of the 120 mg/kg bw dose group and one from the 30 mg/kg bw dose group had excess fluid in the thoracic cavity (due to retardation of development).

Table 6.6-16. Foetal findings

Parameter		Control data		Low dose	Medium dose	High dose
		Historical 1)	Study	30 mg/kg	60 mg/kg	120 mg/kg
External malformations						
Foetuses affected			0	0	0	2
Litters affected			0	0	0	2
Total incidences	F		0	0	0	0.9
	L		0	0	0	8.3
Agnathia, microstomia, anophthalmia	F	0.0 – 0.4 2)	0	0	0	0.4
	L	0.0 – 4.3 2)	0	0	0	4.2
Tail, absent	F	0.0 – 0.4	0	0	0	0.4
	L	0.0 – 4.2	0	0	0	4.2
Visceral findings						
Excess fluid in thoracic cavity 3)	F	*no HCD*	0	0.75	0	3.45
	L	*no HCD*	0	4.2	0	8.33
Skeletal findings						
Foetuses affected			2 4)	2 4)	2 4)	8 4) 5)
Litters affected			2	2	2	5
Supernumerary ribs, one,						
left (%)		**up to 25**	14	20	14	**22****
right (%)		**up to 21**	15	23	15	**21***
Thoracic vertebral centrum either dumbbell shaped or bipartite 4)	F	*no HCD*	1.39	1.46	1.57	6.90
	L	*no HCD*	8.3	8.3	9.1	20.8

F: %foetuses, L: %litter
*/** significantly different from study controls ($p \leq 0.05$ / $p \leq 0.01$),
1) Historical control data range from 1985 - 1988 (within ± 5 years of the study) (31 studies, 763 dams, 8822 foetuses) species rat, strain Wistar/HAN (same species and strain), conducted in the same lab (same main author).
2) Range applies to both findings: Eye – absent and Face – Jaw, lower – absent
3) due to retardation of development
4) lower thoracic vertebral centrum either dumbbell shaped or bipartite , indicative of slight effect on foetal development, correlation with increased incidence of visceral findings and reduced mean foetal weight
5): statistically significant increased incidences in supernumerary ribs, non-ossified cervical vertebrae Nos. 1-6, sacral vertebral arches Nos. 6+7, various phalangeal nuclei and incompletely ossified sternebrae No. 2.

DK RMS Table with overview of results

Dosemg / kg bw/d	Maternal bw gain in pregnancy (and other maternal effects if relevant)	Postimpl loss/fetal death	Litter size	Fetal weight / pup birth weight	Weight of litter	Maternal bw gain vs litter weight	External malformations	Skeletal anomalies
30, gavage, rats, GD 6-15	-	NS (↑ from 4.6% to 6.9%)	-	-	-		-	-
60, gavage, rats, GD 6-15	↓Maternal body weight gain of -15% during treatment and -1% until sacrifice. ↓Feed intake of 7% during treatment gd 6-16. ↑ abs and rel liver weight, 9 and 8 %, respectively.	NS (↑ from 4.6% to 7.6%)	-	-	-	No reduced litter weight, i.e. **cannot explain** the slightly lower BW gain in the dam during pregnancy.	-	-
120, gavage, rats, GD 6-15	↓Maternal body weight gain 60 % (12 g) GD 6-11. ↓Maternal body weight gain of 29 % (12 g) GD 6-15. (NS ↓Maternal body weight gain of 15 % (14 g) GD 6-16). (Exp: 12 g lower bw gain than control during exposure and **14 g lower** than control during pregnancy). ↓ feed intake of 20 % (GD 6-11), ↓ feed intake of 15% during treatment gd 6-16.	**↑ postimplantation loss from 4.6% to 22.1%. Uterus alterations: filled with black/brown fluid**	**↓ litter size from C: 12.0 to Exp: 9.7 fetuses per litter**	**↓ fetal wt from C: 4.7 g to Exp: 4.1 g at GD 21.**	C: 12*4.7 g = 39.77 g Exp: 9.7 * 4.1 g = 56.4 g. Litter weight is **16.6** g lower in exposed than controls.	The reduced litter weight (16.6 g) **fully explains** the reduced maternal bw gain (14 g) during pregnancy		↑ skeletal anomalies from 1.39% to 6.9% (Lower thoracic vertebral centrum either dumbbell shaped or bipartite)

Dosemg / kg bw/d	Maternal bw gain in pregnancy (and other maternal effects if relevant)	Postimpl loss/fetal death	Litter size	Fetal weight / pup birth weight	Weight of litter	Maternal bw gain vs litter weight	External malformations	Skeletal anomalies
	↑relative liver weight 15 %, abs liver weight ↑ 9%.							

Exp: exposure group, C: control group, NS: Not statistical significant

UK-RMS Conclusion

Overall, in a guideline developmental toxicity study in the rat, maternal toxicity (decreased body weight gain, reduced food consumption and increased liver weight) was seen from the mid-dose of 60 mg/kg bw/day. Maternal toxic effects increased in severity at the top dose of 120 mg/kg bw/day. A NOAEL for maternal toxicity of 30 mg/kg bw/day can be identified from this study. Developmental effects were seen only at the top dose of 120 mg/kg bw/day. These consisted of embryo lethality, decreased foetal weight, reduced ossification and an increased incidence of skeletal anomalies. Since there were no adverse effects on development at the next lower dose of 60 mg/kg bw/d, a NOAEL for developmental toxicity at that dose can be identified. These are the same NOAEL values agreed during the first review of tebuconazole.

B.6.6.2.1.1b Discussion and conclusion by DK-RMS:	DK-RMS agrees with this conclusion. The DK-RMS further notes that at a dose of 60 mg/kg bw/d there was no reduction in litter weight, which therefore **cannot** explain the slightly lower BW gain in the dam during pregnancy. In the high dose group (120 mg/kg bw/day) the reduced litter weight (16.6 g) fully explains the reduced maternal bw gain (14 g) during pregnancy. HCD were included in the study report. They were from the same species and strain (Wistar/HAN Rat (Kfm-. WIST, Outbred,SPF Quality)). According to applicant the studies were conducted in the same lab as the Becker study.

c)

Previous evaluation	None: Submitted for the purpose of renewal (Bayer Task Force)

Study ID	B.6.6.2.1.1/03
Study title	HWG 1608 (c.n. Tebuconazole) - Study on maternal toxicity in pregnant rats after oral administration
Matrix ID	20
Experimental dates	April 14 1999 – September 28 1999
Test substance	Tebuconazole (HWG 1608)
Purity (%) **Batch no.**	98.5 / 98.6 278679012
Test animals	SPF-bred Wistar rats of the strain Hsd Cpb:WU
Groups	20/dose
Dose	0 and 120 mg/kg bw on days 6 – 15 post mating.
Route	Oral by gavage
Vehicle	0.5 % Cremophor EL in demineralised water. Total volume applied 10 mL/kg bw
GLP	Yes. With one deviation: The determination of the test compound in maternal plasma and foetal tissue was not performed in compliance with GLP Principles. Study report stated this deviation did not limit the assessment of results.
Guideline	Not specified – investigative study

Deviation	Not applicable
Acceptable	Acceptable as supplementary information
NOAEL	Not applicable – investigative study
Effects at the LOAEL	Not applicable – investigative study

This additional study was performed to investigate further the systemic toxicity of tebuconazole in pregnant rats (data so far only from non-pregnant females) which may have contributed to the developmental toxicity observed in previous studies.

Methods
Groups of 20 inseminated female Wistar rats each were daily treated orally (by gavage) with tebuconazole formulated in 0.5% aqueous Cremophor EL suspension from day 6 to day 15 post-coitum (p.c.) in doses of 0 and 120 mg/kg bw/day. The foetuses were delivered by caesarean section on day 16 p.c. Investigations were performed on general tolerance of the test compound by the females (including determination of general condition, excretory production, feed and water intake, organ weights, histopathology of liver, adrenals, ovaries and pituitary glands) and on the effect of tebuconazole on intrauterine development (including histopathology of placentas). Additionally, maternal plasma and foetal tissue levels of tebuconazole on day 16 p.c. were determined.

Results
Maternal data
Mortality was not affected by treatment with tebuconazole (120 mg/kg bw/day). Two females showed piloerection during the second week of treatment (one female for a single day only). A relationship to treatment- was assumed for these findings since piloerection was not seen in historical control data and feed intake (> 18 % change compared to control for mean feed intake at days 6 – 15) and body weight gain (> 38 % change compared to control for body weight gain) were severely affected (Tables 6.6.-17 and 6.6-18.). A treatment-related decrease in food consumption (Table 6.6-17.), change in water consumption (decrease and increases were seen in treated animals compared to no decreased or increased water consumption observed in controls) and effect on excretion (reduced amount of faeces, light coloured faeces and/or increased urination) was evident. Severe body weight loss occurred in treated females during the first days of treatment (day 6-9 p.c. -228 % change compared to control) and thereafter body weight gain remained impaired, resulting in distinctly reduced body weight gain during treatment and gestation (up to day 16 p.c.), as well as in significantly reduced final body weight, corrected body weight gain and carcass weights.

Table 6.6-17. Mean feed intakes [mg/kw bw/day]

Days (p.c.)	Dose (mg/kg bw/day)	
	0	**120**
0 – 3 *(%)[a]*	17.53	17.31 *(-1.3)*
3 – 6 *(%)[a]*	18.18	18.00 *(-1.0)*
6 – 9 *(%)[a]*	17.69	10.51** *(-40.6)*
9 – 12 *(%)[a]*	19.12	15.61** *(-18.4)*
12 – 15 *(%)[a]*	19.31	13.80** *(-28.5)*
15 - 16 *(%)[a]*	20.35	18.35* *(-9.8)*

* statistically significant difference to control with $p < 0.05$
** statistically significant difference to control with $p < 0.01$
(%)[a] compared to study control

Table 6.6-18. Body weight gain [g mean]

Days (p.c.)	Dose (mg/kg bw/day)	
	0	**120**
6 – 9	8.1	-10.4**

(%)[a]		*(-228.4)*
6 – 16 *(%)[a]*	38.5	15.3** *(-60.3)*
0 – 16 *(%)[a]*	59.1	36.7** *(-37.9)*
Corrected 0 - 16 *(%)[a]*	39.4	19.9** *(-49.5)*

* statistically significant difference to control with $p < 0.05$
** statistically significant difference to control with $p < 0.01$
(%)[a] compared to study control

The liver weight was slightly increased in treated females and a statistically significant increase in relative liver weight was observed (12.6 % change compared to control). These increases were accompanied by histopathological findings, consisting of minimal to moderate hyperplasia of the bile ducts, periportal inflammation and minimal deposition of yellow/brownish pigment. Increased hepatic glycogen accumulation was seen in 3 treated females, while the frequency of focal Kupffer cell proliferation and of focal necrosis was decreased. In the adrenal glands, minimal to slight cytoplasmic vacuolation of the zona fasciculata of the cortex was observed in 8 out of 10 females. Furthermore, minimal vacuolation of cells of the zona glomerulosa occurred in 3 treated females (two of these females also showed increased urination).

Table 6.6-19. Organ weights

Dose (mg/kg bw/day)		**0**	**120**
Liver	absolute (g) relative (%) relative *(%)[a]*	11.346 4.3531	11.876 4.901** *(12.6)*
Ovaries	absolute (g) relative (%) relative *(%)[a]*	0.109 0.0418	0.092** 0.0379 *(-9.3)*
Adrenals	absolute (g) relative (%) relative *(%)[a]*	0.064 0.0244	0.065 0.0270 *(10.7)*
Placentas	absolute (g) absolute *(%)[a]*	0.35	0.36 *(2.9)*

* statistically significant difference to control with $p < 0.05$
** statistically significant difference to control with $p < 0.01$
(%)[a] compared to study control. Relative weight is relative to carcass weight ratio

Table 6.6-19a Selected histopathological findings

Parameter	**Tebuconazole [mg/kg bw/day]**	
	0	**120**
Liver		
Number examined	10	10
Hyperplasia of bile duct (minimal to moderate)	-	**7**
Periportal inflam. infiltration	-	**5**
Pigment periportal	-	**4**
Increased glycogen content	-	**3**
Focal Kupffer cell accumulation	8	2
Focal necroses	3	-

Parameter	Tebuconazole [mg/kg bw/day]	
	0	**120**
Adrenal glands		
Number examined	10	10
Vacuolation, zona fasciculate (minimal to slight)	-	**8**
Vacuolation, zona glomerulosa (minimal)	-	**3**

Findings considered related to treatment with tebuconazole are written in **bold letters**.

Reproduction data
The gestation rate of the females was unaffected by treatment (Table 6.6-20.).

Table 6.6-20. General reproduction data

Dose (mg/kg bw/day)	0	120
Mated females	20	20
Mated females evaluated	20	20
Females with implantations	17	17
% of those mated	85.0	85.0
Mean values Per female with implantation sites		
Corpora lutea	13.5	13.2
Preimplantation loss	1.6	1.1
Implantations	11.8	12.1
Gestation rate		
Females with viable foetuses on day 16 post coitum	17	17
% of females with implantations	100.0	100.0

Developmental toxicity
Post-implantation loss was statistically significantly increased (271 % change compared to control) and correspondingly the mean number of foetuses was decreased (-14 % change compared to control), though without statistical significance, by treatment (Table 6.6-21.). Foetal weight of the exposed groups was decreased by 13 % compared with the controls (Table 6.6-21.).

Table 6.6-21. Foetal data

Dose (mg/kg bw/day)	0	120
Number of females with implantations/viable foetuses	17	17
Number of foetuses *(%)[a]*	11.1	9.5 *(-14.4)*
Post-implantation loss *(%)[a]*	0.7	2.6** *(271.4)*
Foetal weight (g) *(%)[a]*	0.45	0.39** *(-13.3)*

** statistically significant difference to control with $p < 0.01$
(%)[a] compared to study control

Toxicokinetic data
Maternal blood and foetal tissue samples for toxicokinetic investigations were taken 24 hours after the last administration. All samples (maternal plasma and foetal tissue) revealed no tebuconazole at this time, therefore there is no indication that tebuconazole accumulates or persists in the foetus.

Table 6.6-22. Concentration of tebuconazole in maternal plasma and foetus, admin.: day 6 to 15 of pregnancy, samples taken at day 16

Dose (mg/kg bw/day)	0	120
Tebuconazole in plasma, mean [µg/ml(g)]	0.00	0.00
Tebuconazole in foetus, mean [µg/ml(g)]	0.00	0.00

** statistically significant difference to control with $p < 0.01$

(%)[a] compared to study control

DK RMS supplementary table with overview of results

Dose, mg/kg bw/d	Maternal bw gain in pregnancy (and other maternal effects if relevant)	Postimpl loss/fetal death	Litter size	Fetal weight / pup birth weight	Weight of litter	Maternal bw gain vs litter weight	External malformations	Skeletal anomalies
120, gavage, rats, GD 6-15, examination GD 16	↓body weight gain from GD 0 to 16 by 38 % of control value. C: 59.1 g Exp: 36.7 g. Exposed animals show 22.4 g lower bw gain than control from GD 0 to 16. ↓feed intake 18-40 %, ↑relative liver weight 12.6%, ↓abs ovary weight 9.3%. Absolute liver weights showed slight (5%) increase. Histological changes in liver and adrenal.	↑ **at examination age GD 16 (0.7 % in control, 2.6% in exposed)**	(NS ↓ from 11.1 in controls to 9..5 in exposed group)	↓ **fetal wt from 0.45 g to 0.39 g at GD 16**	Wt of litter at GD 16: C: 11.1*0.45 = 5.0 g Exp: 9.5*0.39= 3.7 g Litter weight is **1.29 g lower** than controls.	The reduced litter weight (1.3 g) **cannot explain** the maternal weight gain difference (22.4 g)	-	-

Exp: exposure group, C: control group

UK-RMS Conclusion

This investigative study in the rat clearly revealed significant to severe systemic toxicity of tebuconazole in maternal animals at the 120 mg/kg bw dose level. In addition to generalised effects on body weight, food and water consumption and on clinical signs of toxicity, specific toxic effects were seen in the liver and adrenal gland.

Toxicokinetic investigations revealed that tebuconazole did not accumulate nor persist in females or in foetuses.

Thus besides general toxic effects, liver and adrenal glands are targets for tebuconazole toxicity in pregnant rats; this finding was also evident in short-term (section B.6.3) and chronic (section B.6.5) toxicity tests in non-pregnant rats. The applicant concludes that findings seen in the liver may have contributed to the developmental effects as normal liver function is a prerequisite for normal intrauterine development.

B.6.6.2.1.1c Discussion and conclusion by DK-RMS:	DK-RMS agrees with this conclusion. The DK-RMS further notes that the reduced litter weight (1.3 g) cannot explain the maternal weight gain difference (22.4 g)

B.6.6.2.1.2. Developmental neurotoxicity in rats

a)

Previous evaluation	In DAR (2006) for original approval

Study ID	B.6.6.2.1.2/01
Study title	Developmental neurotoxicity study of technical grade tebuconazole administered orally via diet to Crl: CD®BR VAF/Plus® presumed pregnant rats
Matrix ID	31
Dates	In-life dates: May 1998 to June 1998
Test substance	(HWG 1608) Tebuconazole
Purity (%) **Batch no.**	96.0 - 96.9 603-00l3
Test animals	Sprague-Dawley rats (Crl:CD®BR VAF/Plus®)
Groups	100 mated female rats (25 presumed pregnant rats per dosage group)
Dose	0, 100, 300, or 1000 ppm in the food
Route	Oral, dietary administration
Vehicle	Corn oil (1 % by weight of the diet), acetone (as a solvent in the diet preparation process but was allowed to evaporate), basal diet, no positive control.
GLP	Yes
Guideline	US-EPA, OPPTS 870.6300; US-EPA, Pesticide Assessment Guidelines, Subdivision F - Hazard Evaluation: Human and Domestic Animals, Addendum 10, Neurotoxicity
Deviation	None – however the study was not conducted in accordance with OECD test guideline number 426.
Acceptable	Acceptable
Supplemental report	Includes analytical data on dose preparation, positive control data in rats, including historical control data, and neuropathology validation.
NOAEL	Maternal toxicity: 300 ppm (22 and 41.3 mg/kg bw/day for female during gestation and lactation respectively). Developmental toxicity: 300 ppm (41.3 mg/kg bw/day for female during lactation) Developmental neurotoxicity: 1000 ppm (65 and 125.4 mg/kg bw/day for female during gestation and lactation respectively) (the top dose group).
Effects at the LOAEL	Maternal toxicity: mortality, reduction in body weight and feed consumption, and prolonged gestation in the 1000 ppm dosage group (65 and 125.4 mg/kg bw/day for female during gestation and lactation, respectively) (the top dose group). Developmental toxicity: mortality, decreased number of live born (-6% compared to control), decreased viability index (-6%), reduction in pup body weight and body weight gains, reduction in pup absolute brain weight, delay in vaginal patency and decreased cerebellar thickness in the 1000 ppm dosage group (125.4 mg/kg bw/day for female during lactation) (the top dose group).

Methods
The neurotoxic effects in offspring after exposure to Tebuconazole *in utero* and during the neonatal period through postpartum day 11 was tested in Wistar rats continually beginning on gestation day 6 and continuing through gestation day 24 (rats that did not deliver a litter) or through lactation day 11 (dams that delivered a litter). Groups of 25 pregnant females (F_0 generation) were fed diets containing 0, 100, 300 or 1000 ppm 96.0 % - 96.9 % pure tebuconazole.

Dose: Feeding *ad libitum* with diets containing 0, 100, 300, or 1000 ppm tebuconazole resulted in the following daily test substance intakes (based on feed consumption, bodyweight and analytical results):

Table 6.6-23. Study design and doses

Dose per animal [mg/kg bw/day]	Gender	Concentration in diet [ppm]			
		0	100	300	1000
Gestation (days 6 – 21)	Female	0	8.8	22.0	65.0
Lactation (days 1 – 12)	Female	0	16.3	41.3	125.4

During the exposure and post-exposure periods, the rats were examined for signs of autonomic dysfunction, abnormal postures, abnormal movements or abnormal behaviour patterns, and unusual appearance daily. The dams were evaluated for duration of gestation, litter size, live litter size and pup viability at birth. Maternal behaviour of the dams was evaluated daily. Body weights and feed consumption values were recorded on gestation day 0, daily during the exposure and post-exposure periods and on the day of sacrifice (body weight only).

Pups (F_1 generation) were observed for viability at birth, and at least twice daily during the pre-weaning and post-weaning periods. Clinical observations were recorded daily during the pre-weaning period and weekly during the post-weaning period. Extended clinical observations were recorded for the rats in Subset 4 weekly during the post-weaning period. Rats assigned to Subsets 2 and 3 were examined for gross signs of toxicity when they were weighed or removed from their cages for behavioural testing. Bodyweights were recorded on post-partum days 1, 5, 8, 12, 14, 18 and 22, weekly during the post-weaning period and at sacrifice. Feed consumption values were recorded weekly during post-weaning. Female rats were examined for the age of vaginal patency beginning on post-partum day 28, and male rats were evaluated for the age of preputial separation beginning on post-partum day 39.

On post-partum day 5, litters were reduced to five male and five female pups per litter for continued observation. These pups were assigned to each Subset as follows: post-partum day 12: brain weights and neurohistology examinations (Subset l), passive avoidance and watermaze testing (Subset 2), motor activity and auditory startle habituation (Subset 3), brain weights and neurohistology examinations (Subset 4), and one pup per sex used to standardize litter size to eight pups per litter on post-partum days 12 to 22 (Subset 5). Additionally, six rats of each sex and dosage group in Subsets l and 4 were selected for neurohistological examination. The F_1 generation pups/rats selected for continued observation were sacrificed after completion of all post-weaning behavioural evaluations (on post-partum days 12, 87 to 90, 90 to 93, 83 and 22 for the respective subsets). All pups/rats were necropsied; gross lesions were retained

Results
Parental animals
Clinical signs and mortality
The incidence of alopecia during gestation and lactation were significantly increased in the 1000 ppm group. The mortality rate was not affected by treatment; one female in the 100 ppm group and two females in the 1000 ppm group died or were moribund, during the peri-partum period, however, this was by the UK-RMS not considered treatment related. The DK-RMS consider the two maternal deaths/moribund sacrifices (GD 22 or 23) were likely related to dystocia, and further notes that prolonged gestation was observed in this dose group.

Table 6.6-24. Mortality

Mortality	Nominal Dose[a] [ppm]							
	0		100		300		1000	
	M	F	M	F	M	F	M	F
Generation - P	-	-	-	-	-	-	-	-
Generation – F_0	-	-	-	1	-	-	-	2

Generation – F_1	-	-	-	1	-	-	1	-

Body weight and food intake

Gestation: Maternal body weight was statistically significantly reduced during gestation at 1000 ppm (- 16 % change compared to control on days 0 – 21) (Table 6.6-26). This observation was accompanied by a reduction in food consumption at 1000 ppm (statistically significant but < 10 % change compared to control) (Table 6.6-25).

Lactation: Maternal body weight was also affected during lactation at 1000 ppm, however an increase in body weight change was seen (21 % change compared to control) at this dose level) (Table 6.6-27). This observation was not accompanied by a biologically significant change in food consumption.

The effects on food consumption and body weight change were not dose-dependent and were not considered treatment-related.

Table 6.6-25. Food consumption (% change compared to control)

	Nominal Dose[a] [ppm]			
	0	**100**	**300**	**1000**
Gestation	-	*2*	*1*	*-5**
Lactation	-	*-3*	*-1*	*-2*

*/** significantly different from study controls *($p \leq 0.05$ / $p \leq 0.01$)*

Table 6.6-26. Body weight changes – gestation (mean ± standard deviation)

Gestation	**Nominal Dose[a] [ppm]**							
	0		**100**		**300**		**1000**	
Rats tested [n]	25		25		25		25	
Rats pregnant [n]	25		24		24		23	
Body weight change [g] Day 0-21	+152.6	±24.3[b]	+162.9	±20.2[b]	+158.0	±17.1[b]	+127.9	±23.3**[b]
(%)[a]			*(7)*		*(4)*		***(-16)***	

(%)[a] percent change compared to control
*/** significantly different from study controls *($p \leq 0.05$ / $p \leq 0.01$)*
[a] Doses occurred on day 6 of gestation through day 11 of lactation
[b] Excludes values for dams that delivered or were in the process of delivering

Table 6.6-27. Body weight changes – lactation (mean ± standard deviation)

Lactation	**Nominal Dose[a] [ppm]**							
	0		**100**		**300**		**1000**	
Rats tested [n]	25		25		25		25	
Rats pregnant [n]	25		24		24		23	
Delivered litters	25		24		24		21[b]	
Body weight change [g] Day 0-21	+49.9	±19.2[cd]	+46.5	±22.9[de]	+51.2	±18.2[d]	+60.3	±25.2[d]
(%)[a]			*(-7)*		*(3)*		***(21)***	

(%)[a] percent change compared to control
[a] Doses occurred on day 6 of gestation through day 11 of lactation
[b] Excludes values for dams that die or were moribund sacrificed
[c] Excludes values for dam 7539 which delivered one additional pup on lactation day 2
[d] Excludes values for dams that were sacrificed on day 12 of lactation
[e] Excludes values for dam 7506 which was found dead on lactation day 17

Delivery observations

The duration of gestation was slightly (2.2 % change compared to control) but statistically significantly increased at 1000 ppm. No other treatment-related effects were recorded.

Table 6.6-28. Delivery observations in the F_0 generation

	Nominal Dose [ppm]			
	0	**100**	**300**	**1000**
Rats tested [n]	25	25	25	25
Rats pregnant [n]	25	24	24	23
Delivered litters	25	24	24	21[a]
Duration of gestation (mean days) *(%)[a]*	22.5 -	22.6 *(0.4)*	22.7 *(0.9)*	23.0** *(2.2)*
Gestation index *(%)[a]*	-	±0	±0	-8.7
Dams (stillborn pups) [n]	2	1	2	5
Dams (no live born pups) [n]	0	0	0	0
Pathology	Same (few) findings in all groups – not related to dosing			
Histopathology examination (incidence)	Reported findings are all scattered and/or incidental or common in ~~Wistar~~ Sprague-Dawley rats and not related to dosing			
Clinical examination	Clinical observations were considered unrelated to the test substance (not dose-dependent)			

(%)[a] percent change compared to control
*/** significantly different from study controls *($p \leq 0.05$ / $p \leq 0.01$)*
[a] Excludes values for dams that die or were moribund sacrificed

Litter observations
An increase in mortality was evident at 1000 ppm. At this dose the number of stillborn pups (7 stillborn pups at 1000 ppm compared to 2 in the control; a 250 % increase compared to control) and the number of pups found dead or presumed cannibalized on lactation days 2 to 5 were increased. In addition the number of live born pups was reduced (an average 13.1 live born pups per litter at 1000 ppm compared to 13.9 live born control pups per litter; -6 % change compared to control). In the top dose group pup food consumption was statistically significantly increased and body weight gain was statistically significantly decreased, however < 10 % change compared to control was observed in these two parameters. The Viability Index was also statistically significantly reduced at 1000 ppm (-6.3 % change compared to control) in the F_1 generation.

Table 6.6-29. Litter observations in the F_1 generation

	Nominal Dose [ppm]							
	0		**100**		**300**		**1000**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Food consumption *(%)[a]*	-	-	1	1	-1	1	3*	5**
Body weight gain *(%)[a]*	-	-	0	-4	-4*	-1	-8**	-5*
Pathology	Same (few) findings in all groups – not related to dosing							
Histopathology examination (incidence)	Reported findings are all scattered and/or incidental or common in Wistar rats and not related to dosing							
Clinical examination	Clinical observations were considered unrelated to the test substance (not dose-dependent)							
Viability index (%)[b] *(%)[a]*	97.9 -		98.8 *(0.9)*		98.2 *(0.3)*		91.7** *(-6.3)*	
Lactation index (%)	100		100		100		99.4	

(%)[a] percent change compared to control
*/** significantly different from study controls *($p \leq 0.05$ / $p \leq 0.01$)*
[b] number of live pups on lactation day 5 divided by the number of live born pups on lactation day 0

Neurobehavioral: There were no effects on motor activity, auditory startle habituation or tests of learning and

memory (passive avoidance and water maze) in the offspring at any dose level.

Neuropathology: In offspring, gross and microscopic measurements, including histopathology of the brains from the different dosage groups did not show a dose-dependent relationship. The animals in the high-dose group showed at post-partum day 12 decreased brain weights and decreased cerebellar thickness. These decreases were not accompanied by histopathological findings and were considered the secondary consequence of the reduced body weight gains.

Table 6.6-30. Terminal body weights, brain weights and ratios (%) of brain to terminal body weights F_1 generation (mean ± SD)

Dose [ppm]	**0**	**100**	**300**	**1000**
Males				
Male rats tested	20	20	19	19
Terminal body weight [g]	24.8 ± 2.3	23.8 ± 2.1	23.7 ± 2.2	19.0 ± 2.2**
Brain weight [g]	1.359 ± 0.060	1.301 ± 0.061*	1.317 ± 0.061	1.153 ± 0.089**
Brain ratio [%]	5.526 ± 0.455	5.501 ± 0.351	5.590 ± 0.440	6.094 ± 0.497**
Females				
Female rats tested	20	20	19	16
Terminal body weight [g]	24.4 ± 2.3	22.4 ± 2.4**	21.9 ± 1.7**	17.8 ± 2.3**
Brain weight [g]	1.325 ± 0.061	1.267 ± 0.070*	1.273 ± 0.057*	1.115 ± 0.101**
Brain ratio [%]	5.466 ± 0.437	5.706 ± 0.426	5.825 ± 0.424*	6.302 ± 0.430**

* statistically significant difference from control $p \leq 0.05$
** statistically significant difference from control $p \leq 0.01$

UK RMS Conclusion
This developmental neurotoxicity study of tebuconazole in mated female Sprague-Dawley rats were in accordance with US-EPA guidelines. In the 1000 ppm dams, mortality, prolonged gestation, alopecia (localized), decreased body weight (gestation) and decreased feed consumption (gestation and lactation) were noted. F_1 pups showed mortality, decreased number of live born, decreased viability index, developmental delay (vaginal patency), decreased body weight/gain, decreased absolute brain weight (post-partum day 12 and adult) and decreased cerebellar thickness at 1000 ppm. The delay in vaginal patency, the reduced brain weight and the decreased cerebellar thickness were considered the secondary consequence of the reduced body weight gains.
The 1000 ppm dosage level was considered to be an excessively toxic dosage for the F_1 offspring (mortality, reduction in pup body weight and body weight gains). By approximately day 80 post-partum, the body weight had completely recovered in the females but was still reduced (89 % of the control group value) in the males. The brain weights had shown an incomplete recovery (90 % to 93 % of the control group values) in both sexes.

The NOAEL for maternal toxicity in this study was 300 ppm (22 and 41.3 mg/kg bw/day for female during gestation and lactation respectively), based on mortality, reduction in body weight and feed consumption, and prolonged gestation in the 1000 ppm dosage group (65 and 125.4 mg/kg bw/day for female during gestation and lactation respectively) (the top dose group).

The NOAEL for developmental toxicity was 300 ppm (41.3 mg/kg bw/day for female during lactation), based on mortality, reduction in pup body weight and body weight gains, reduction in pup absolute brain weight at post-partum day 12, delay in vaginal patency and decreased cerebellar thickness in the 1000 ppm dosage group (125.4 mg/kg bw/day for female during lactation) (the top dose group). These are the same NOAEL values agreed during the first review of tebuconazole.

Tebuconazole did not cause any specific developmental neurotoxicity in the offspring when administered to the dams during gestation and lactation at dietary concentrations up to and including 1000 ppm (the top dose).

B.6.6.2.1.2a Discussion and conclusion by DK-RMS:	DK-RMS partly agrees with this conclusion, but finds that some important information is lacking in the UK-RMS conclusion. Two females in the 1000 ppm group died or were moribund, during the peri-partum period (GD 22 or 23) and these findings were likely related to dystocia, an adverse effect on fertility caused by tebuconazole exposure. This corresponds with the observed prolonged gestation length in this dose group.

	The observed delays in vaginal patency (day 31.6 in control females vs. 33.2 day in high dose females), was by the UK RMS considered as a secondary effect to the reduced body weight gains, but could also be a sign of endocrine disruption. The reductions in maternal body weight gain during pregnancy is not considered to influence the ability to give birth, and effects are thus not secondary to systemic toxicity. Changes in maternal body weight are generally not considered to influence gestation length, as determined from studies on feed restriction (Carney et al. 2004). Additionally, the DK-RMS notes that reduced brain weights can be considered adverse, even in the presence of reductons in body weight. And that adverse effects on the brain are supported by the decreased cerebellar thickness. These effects may be considered signs of developmental neurotoxicity.

b)

Previous evaluation	In DAR (2006) for original approval

Study ID	B.6.6.2.1.2/01
Study title	The effects of perinatal tebuconazole exposure on adult neurological, immunological, and reproductive function in rats
Matrix ID	55
Test substance	Tebuconazole
Purity (%) **Batch no.**	97.4 No information available
Test animals	Pregnant Sprague-Dawley rats (strain: Tac: N(SD)fBR)
Groups	≥15/dose (assigned by stratified randomisation)
Dose	0, 6, 20, or 60 mg/kg from gestational day 14 to postnatal day 7; the pups were then dosed daily at the same levels from postnatal day 7 – 42
Route	Oral by gavage
Vehicle	0.7 % methylcellulose solution
GLP	No
Guideline	Not in accordance with any testing guideline
Deviation	Not applicable
Acceptable (UK-RMS)	No- not reliable. The neuropathological findings were withdrawn as artefacts by the authors. This questions the validity of the other findings reported in this study. It is also noted that no maternal toxicity was reported even at the top dose of 60 mg/kg bw/d, which is inconsistent with all the other available regulatory developmental and RDT studies in rats.
Reliability by DK-RMS	Reliable with restrictions. Withdrawal of neuropathological findings does not question the validity of the reproductive data. With regards to a lack of maternal toxicity in this study it its noted that doses around 50-60 mg/kg seem to be the treshold where maternal toxicity manifests, and biological varation can affect the results in a single study.
NOAEL	20 mg/kg bw/day
Effects at the LOAEL	Maternal weight gain during pregnancy reduced from 87.8 g (control) to 74.0 g (60 mg/kg bw/d group). The reduced litter weight partly explains the reduced maternal weight gain. Developmental: Decreased pup viability and body weights, altered learning in the spatial cognitive task and a number of organ weight changes at the highest dose tested (60 mg/kg bw/day). Tendency towards decreased number of live pups on PND 0. The number of dead pups per litter was significantly increased. At birth, the the pup weight was reduced.

Methods
Sprague-Dawley dams (≥15/dose) were administered tebuconazole (0, 6, 20, or 60 mg/kg bw) by oral gavage daily from gestational day 14 to postnatal day 7; the pups were then dosed daily by direct gavage administration at the same levels from postnatal day 7 - 42.

Separate groups of rats (one male and one female from each litter) were used for testing of immunological parameters, neurobehavioral testing using a screening battery of functional tests, and cognitive evaluations. Other groups of rats were evaluated for reproductive development and function, while yet others were sacrificed at the end of the dosing period for histological analyses of major organs systems, including neuropathological assessments.

Results

Pup viability and body weights were decreased in the highest dose group. Tendency towards decreased number of live pups PND 0 (p=0.07) . Table below inserted by DK-RMS.

TABLE 2
Developmental Indices Following Tebuconazole Treatment

	Tebuconazole dose (mg/kg/day)			
	0	6	20	60
Neonate				
No. of litters	35	30	34	37
PND0				
No. of live/litter	11.2 ± 0.6	10.7 ± 0.6	10.9 ± 0.5	9.7 ± 0.8
No. of dead/litter	0.4 ± 0.2	0.2 ± 0.1	0.6 ± 0.3	2.2 ± 0.6*
Eye opening (day)				
Right	14.0 ± 0.1	14.1 ± 0.1	13.8 ± 0.1	13.7 ± 0.1
Left	14.0 ± 0.1	14.1 ± 0.1	13.8 ± 0.1	13.6 ± 0.2
PND1 anogenital distance (mm)				
Male	3.7 ± 0.1	3.8 ± 0.1	3.7 ± 0.1	3.7 ± 0.1
Female	1.3 ± 0.02	1.3 ± 0.03	1.3 ± 0.02	1.3 ± 0.03
Postweaning				
No. of litters	25	25	25	17
No. of female rats	88	91	80	70
Vaginal opening (day)	35.9 ± 0.3	35.7 ± 0.5	34.0 ± 0.3*	34.7 ± 0.4
No. of litters	18	21	23	15
No. of male rats	36	42	46	41
PS (day)	41.2 ± 0.25	40.9 ± 0.30	41.2 ± 0.24	41.2 ± 0.26

Note. All data are presented as mean ± SEM.
*Indicates statistically significant compared to control.

In sheep RBC-immunized high-dose rats, spleen weights and cellularity were increased, and the ratio of cell types was altered at 60 mg/kg bw/day (the tope dose) compared to controls. There were, however, no biologically significant changes in the immune function of these rats. One month after the end of dosing, acquisition of learning the platform location in a water tank (i.e. Morris water maze) was impaired in the high-dose group. However, there was no effect on recall of the position during a free-swim trial.

At necropsy on postnatal day 46 or l52, kidney, liver, and spleen weights were altered by tebuconazole treatment, but a dose-response relationship was not clear for most organs; only decreased kidney and increased liver weights were consistent in both sexes (statistically significant at high dose). On PND46 relative liver weight was significantly increased in male and female F1 offspring (60 mg/kg bw/day group). No effects were seen on body weight.

In adult male F1 offspring there was a significant reduction in absolute epididymis weight (17%) at 60 mg/kg bw/day group. Nominal dose-dependent reductions in epididymis weight was seen at both lower doses. Male body weight was reduced (9.5%) in 60 mg/kg bw/day group. In adult pregnant F1 offspring, corrected bodyweight (terminal bodyweight minus uterine contents) was significantly increased in adult pregnant F1 females in the highest dose group.

Histological analyses were generally unremarkable outside of the brain. Neuropathological evaluations revealed pyknotic (cells across hippocampal cell fields) in animals of all tebuconazole treatment groups, with the highest incidence in the 20 and 60 mg/kg/day dose groups, coincident with cell loss within pyramidal cell layer of CA3-4 cell fields of the hippocampus and layer of the neocortex. These neuropathological findings have later been withdrawn as artefacts by the authors (see Barone & Moser (2004)).

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LETTER TO THE EDITOR

To the Editor:

Our paper entitled "The Effects of Perinatal Tebuconazole Exposure on Adult Neurological, Immunological, and Reproductive Function in Rats" (Moser *et al.,* 2001) was part of a multidisciplinary project to evaluate long-term effects of developmental exposure to several different pesticides and to compare those effects across multiple forms of toxicity (neuro-, immuno-, and reproductive toxicity). We reported that tebuconazole produced impaired cognition, neuropathology, and altered organ weights, whereas immune and reproductive function were not affected. Questions arose regarding the finding of a treatment-related increase in the number of dark staining neurons and cell loss. To address these questions, we convened a group of expert neuropathologists to re-examine the histological sections of brain from this study. Following this re-examination, we concluded that the dark staining neurons were not pyknotic cells but were artifacts related to fixation and handling, and not a direct result of treatment. Based on this new interpretation, we now withdraw all neuropathological conclusions in the paper, and their implications or relevance to any other findings. The neurobehavioral, immunological, and general toxicity findings of the paper stand unchanged.

This letter to the editor does not necessarily reflect EPA policy.

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REFERENCE

Moser, V. C., Barone, S. Jr., Smialowicz, R. J., Harris, M. W., Davis, B. J., Overstreet, D., Mauney, M., and Chapin, R. E. The Effects of Perinatal Tebuconazole Exposure on Adult Neurological, Immunological, and Reproductive Function in Rats. *Toxicol. Sci.* **62,** 339–352.

Thus, perinatal exposure to tebuconazole produced cognitive (learning) deficits in rats at a dose (60 mg/kg bw/day) which also caused mortality and reduced body weights, but did not alter immunological or reproductive function, including ano-genital distance (AGD).

DK RMS Table with overview of results

Dose, mg/kg bw/d	Maternal bw gain in pregnancy (and other maternal effects if relevant)	Postimpl loss/fetal death	Litter size	Fetal weight/ pup birth weight	Weight of litter	Maternal bw gain vs litter weight	External malformations	Skeletal anomalies
6, gavage, rats, GD 14 to PND 7	-	-	-	-	-		ND	ND
20, gavage, rats, GD 14 to PND 7	-	-	-	-	-		ND	ND
60, gavage, rats, GD 14 to PND 7	The maternal bw was decreased by 13.3 g on GD 21 and bw gain was decreased by **13.8 g** (Exp: weight 341.5 ± 5 g, bw gain 74 ± 2.8 g, controls: weight 354.8 ±6.3 g, bw gain 87.8 ± 3.3 g). Maternal bw at birth is not presented, but would be useful for	**↑ from 0.4 dead/ litter to 2.2 dead/ litter**	- C: 11.6 pups/ litter Exp: 11.9 pups/ litter	**Pup bw at birth: ↓ C: 7.6 g Exp: 6.6 g**	C: 11.6 * 7.6 g = 88,16 g. Exp: 11.9 *6.6 g = 78.54 g. Litter weight is **9.6 g lower** in exposed than controls.	The reduced litter weight (9.6 g) **partly explains** the reduced maternal weight gain (13.8 g) on GD 21	ND	ND

Dose, mg/kg bw/d	Maternal bw gain in pregnancy (and other maternal effects if relevant)	Postimpl loss/fetal death	Litter size	Fetal weight/ pup birth weight	Weight of litter	Maternal bw gain vs litter weight	External malformations	Skeletal anomalies
	comparison with litter weight differences.							

Exp: exposure group, C: control group

UK RMS Conclusion

In this non-guideline study reported in a peer-reviewed article perinatal exposure to tebuconazole produced reduced pup viability, reduced pup body weights, changes in the weights of kidney and liver and altered learning in a spatial cognitive task at the highest dose tested (60 mg/kg bw/day). In contrast, there were no overall effects on the immunological or reproductive systems. A NOAEL of 20 mg/kg bw/day was identified during the first review of tebuconazole. The UK RMS questions the reliability of this study as explained in the introductory table.

B.6.6.2.1.2b Discussion and conclusion by DK-RMS:	The DK-RMS agrees with the conclusions regarding observed effects and NOAEL setting, but not with the UK-RMS conclusion that the study is unreliable due to redrawn neurotoxicity results and a lack of maternal toxicity. The systemic toxicity seen in other studies usually comprises of decreased maternal body weight gain. Although not statistically significant there was a nominal reduction in maternal body weight and body weight gain during pregnancy. A lack of statistically significant maternal toxicity at 60 mg/kg bw/day is not a reason to disregard the study. In addition, doses around 50-60 mg/kg bw/d seem to be the treshold where this effect manifests, and biological varation can affect the results in a single study. Here, the reduced maternal weight can partly be explained by fewer and smaller pups indicating minimal or absence of systemic toxicity.

c)

Previous evaluation	In DAR (2006) for original approval

Study ID	B.6.6.2.1.2/03
Study title	Tebuconazole - An assessment of its potential to produce developmental neurotoxicity
Test substance	Not applicable – this study examined the results of two studies for developmental neurotoxicity (DNT) (B.6.6.2.1.2/01and B.6.6.2.1.2/01)
Purity (%) Batch no.	
Test animals	
Groups	
Dose	
Route	
Vehicle	
GLP	
Guideline	
Deviation	
Acceptability	
NOAEL	
Effects at the LOAEL	

Methods

An abbreviated neuropathology peer review was performed by an independent expert (Dr. Garman) on slides of rat brain archived in the laboratory of Dr. Stanley Barone Jr. (Neurotoxicology Division of the USEPA). All available slides from rats in the high dose and control groups were examined with knowledge as to treatment group (based on an animal identification list provided by Dr. Barone).

Although the slides were examined for the presence of any neuropathologic alteration, particular emphasis was placed on scoring the numbers of dark neurons (referred to by Dr. Barone as 'pyknotic') in the hippocampus. The hippocampi were also examined for the presence of any evidence of neuron loss.

Results
The dark neurons are considered to represent the result of handling artefact within these immersion fixed brains. Furthermore, semi-quantitative scoring of the numbers of these dark neurons failed to indicate any treatment-related differences in their numbers based on examination of the control and high dose groups.

Overall, therefore, the concerns and doubts initially expressed above with respect to the validity of the findings in the publication by study B.6.6.2.1.2/01 were confirmed by the outcome of this slide review.

The quality of the tissues was reported to be "clearly inadequate for critical assessment of neuropathology and the reported findings were not consistent with the exposure (no dose-response) or period of time (100 days) that elapsed between the termination of exposure and collection of brain tissue."

UK RMS Conclusion
Based on all of the available information, including that provided in B.6.6.2.1.2/01, the review of the slides from that study, and the absence of neuropathology in Bayer's Developmental Neurotoxicity (DNT) study (B.6.6.2.1.2/01), it could be concluded that no evidence is found that exposure to tebuconazole during development produces neuropathology at any dose level.

This was further confirmed in a peer review in August 2003, by six independent pathologists; resulting in a retraction "Letter to the Editor" published in 2004 (S. Barone, Jr., and V.C. Moser, Toxicological Science 77, 183, 2004).

B.6.6.2.1.2c Discussion and conclusion by DK-RMS:	Overall, the DK-RMS agrees with this conclusion and do not consider tebuconazole a developmental neurotoxicant. The decreased brain weight and cerebellar thickness in high dose animals in B.6.6.2.1.2/01 may be treatment related but no by effects were observed on neurobehaviour or histopathology of the brain.

B.6.6.2.1.3. Developmental toxicity study by dermal administration to rats

Two developmental toxicity studies by dermal administration in the rat were described in the original DAR (2006) (B.6.6.2.1.3/01 and B.6.6.2.1.3/02). No new developmental toxicity studies by dermal administration have been submitted.

a)

Previous evaluation	In DAR (2006) for original approval

Study ID	B.6.6.2.1.3/01
Study title	HWG 1608 – Study for embryotoxic effects on rats after dermal administration
Matrix ID	22
Test substance	(HWG 1608) Tebuconazole
Purity (%) **Batch no.**	97.4 16012/86
Test animals	Female WISW (SPF Cph) rats – mated (until sperm was found in vaginal smear)
Groups	25/dose
Dose	0, 100, 300 or 1000 mg/kg bw/day on day 6-15 post mating
Route	**Dermal** – applied to shaved skin on gauze dressing with aluminium foil base for six hours/day
Vehicle	1% aqueous Cremophor EL. The volume applied was 2 mL/kg bw
GLP	Yes
Guideline	US-EPA 83-3 (1984) complies with OECD TG 414. Both the EPA and the OECD guidelines have been revised (OECD guideline 414 in 2001) with extension of the dosing period to cover more than the organogenesis period and a few minor changes.
Deviation	Although not a deviation from the guideline available at the time, the foetuses are very small

	as the Caesarean operation was done one day earlier than normal. The following deviations from the OECD-Guideline 414 (2001) occurred: - Dosing was performed during the period of organogenesis. - Caesarean section was performed one day too early resulting in very small foetuses. - Less than 50% of the foetuses were examined for visceral alterations. - Reporting is not sufficient – no raw data presentation. No corpora lutea data were given. No measurement of crown-rump length. - Numbers of pre- and post-implantation losses were not given in the report but were re-evaluated based on raw data. The deviations are not found to totally compromise the results with respect to embryo- or developmental toxicity
Acceptable	Acceptable, with restrictions.
NOAEL	Maternal and developmental NOAEL: 1000 mg/kg bw/day (the highest dose tested).
Effects at the LOAEL	Not applicable. No systemic effects recorded.

Methods
In accordance with the EPA Guidelines 83-3, "Teratology Study" and OECD Principles of Good Laboratory Practice groups of 25 mated female Wistar rats were administered daily dermal doses of pure (97.4 %) tebuconazole on days 6 to 15 of gestation. The substance was suspended in 1 % aqueous Cremophor EL emulsion, which was applied evenly to the shorn skin of the backs and covered with occlusive bandage. Six hours after the application the bandage was removed and the skin was washed with lukewarm water. The animals were inspected at least once daily for mortality, clinical signs, changed appearance and behaviour. The animals were weighed at regular intervals and food consumption was recorded. On day 20 of gestation Caesarean section was performed on all dams. The following examinations were performed: necropsy findings on dams, determination of implantation sites, number of corpora lutea and of uterus weight. Determination of number of live and dead foetuses or embryos, determination of the sex and weight of each live foetus, and recording of number of runts, and determination of individual placenta weights were performed. Examination of all foetuses for external malformations, a number of the foetuses for visceral malformations (modified Wilson's method) and the rest of the foetuses for bone alterations (alizarin red S stained) after exenteration and appraisal of the abdominal and thoracic organs were performed.

Results
None of the parameters examined, whether with respect to gestation index, reproduction toxicity or teratogenicity as well as toxicity to the dams were significantly different between any dose group and the controls.

Table 6.6-31. Appearance

Dose group (mg/kg)	A	B	C	D	E
0	-	-	-	10	14
100	-	-	1	6	16
300	-	1	-	7	18
1000	1	-	-	8	18

A ear swollen (ear mark)
B hydronephrosis
C bloody anal discharge
D intestinal worms
E wound in flank, neck, thoracic and/or dorsal area

Table 6.6-32. Weight gains of pregnant animals (mean, g)

Dose group (mg/kg)	Administration period	Total gestation
0	14.3	75.0
100	13.8	78.1
300	16.5	80.4
1000	16.2	77.5

Table 6.6-33. Insemination and fertilisations

Dose group (mg/kg)	Inseminated females	Fertilised total	Females (% of inseminated)	Pregnant total	Females (% of fertilised)
0	25	24	96.0	24	100
100	25	23	92.0	23	100
300	25	22	88.0	22	100
1000	25	23	92.0	23	100

Table 6.6-34. Malformations

Dose group (mg/kg)	Dam No.	Foetus No.	Malformation
0	2322	118	Hydronephrosis left
	2325	164	Microphthalmia left
	2330	190	Dysplasia of scapula and long bone
	2369	552	Microphthalmia, cleft palate, oedema, closed abdominal fissures
100	2371	586	Hydronephrosis right
300	2376	639	Microphthalmia right
		629	Humerus dysplasia
	2387	724	Microphthalmia right
1000	2305	25	Cryptorchismus
	2404	923	Fused ribs, asymmetric vertebra

Many of the animals were infected with intestinal worms and many of the animals had wounds in combination with the application sites - approximately same number in dosed groups and controls (data not included).

UK RMS Conclusion
Under the conditions of this limited EPA Guideline 83-3, "Teratology Study" dermal applications of tebuconazole for 6 hours/day on day 6-15 of gestation were not toxic at any of the tested doses to any of the parameters examined in the study. There was no indication of a developmental effect for tebuconazole up to and including 1000 mg/kg bw/day (the highest dose tested). Under the tested conditions the dermal NOAELs for both maternal effects and developmental effects were 1000 mg/kg bw/day. These are the same NOAEL values agreed during the first review of tebuconazole.

B.6.6.2.1.3a Discussion and conclusion by RMS-DK:	DK RMS agrees with this conclusion and notes that a dermal dosing most likely results in very different internal concentrations of the active compound than oral dosing, due to ADME differences related to the exposure route.

b)

Previous evaluation	In DAR (2006) for original approval

Study ID	B.6.6.2.1.3/02
Study title	Limit test for embryotoxicity (including teratogenicity) with HWG 1608 technical (c.n. Tebuconazole) in the rat (dermal application)
Matrix ID	21
Test substance	(HWG 1608) Tebuconazole
Purity (%) **Batch no.**	96.2 or 95.8 816196048 - two included analytical reports (TOX 3175-00) (TOX 3175-01) gave different results
Test animals	Mated female WIST HanIbm (SPF) rats (until spermatozoa in vaginal smear or a vaginal plug had been observed)
Groups	25/dose
Dose	0 or 1000 mg/kg bw/day
Route	**Dermal** application (applied to shaved skin with occlusive dressing for six hours once daily)

Vehicle	1% aqueous Cremophor EL emulsion. During most of the study period 2.5 mL/kg bw/day for all animals (see below - Methods).
GLP	Yes
Guideline	OECD TG No. 414 (1981); US-EPA paragraph 163.83-3 (1984) Both the EPA and the OECD guidelines have been revised (OECD guideline 414 in 2001) with extension of the dosing period to cover more than the organogenesis period and a few minor changes.
Deviation	No deviations in relation to the guidelines mentioned in the report. The following deviations from the OECD-Guideline 414 (2001) occurred: - Dosing was performed during the period of organogenesis only - Although this limitation could have affected the outcome of the study, there are other pre-natal and peri-natal studies where exposure during the period after organogenesis has been investigated.
Acceptable	Acceptable
NOAEL	Systemic maternal toxicity and developmental: 1000 mg/kg bw/day (the highest dose tested).
Effects at the LOAEL	Not applicable. No systemic effects recorded.

Methods
In accordance with OECD guideline 414 "Teratogenicity" and EPA Guideline 83-3, "Teratology Study" and OECD Principles of Good Laboratory Practice, 2 groups of each 25 mated female Wistar rats applied daily doses of pure tebuconazole (95.8 - 96.2 %) on days 6 to 15 of gestation. The substance (dose level not adjusted to the content of active ingredient) was suspended in 1% aqueous Cremophor EL emulsion (the testing laboratory tried to use a concentration of 80 % w/v, but this turned out to be too viscous within very short time, so instead a concentration of 40 % w/v was used after 1 (5 rats) or 2 days (7 rats). For the rest of the group the concentration had been 40 % w/v from the start of dosing), which was applied evenly to the shorn skin of the backs and covered with occlusive bandage. Six hours after the application the bandage was removed and the skin was rinsed with lukewarm tap water. The animals were inspected at least twice daily for mortality, systemic clinical signs, changed appearance and behaviour. The animals were weighed daily and food consumption was recorded at regular intervals. On day 21of gestation the animals were sacrificed and caesarean sections were performed. The following examinations were performed: gross macroscopic findings on dams, determination of implantation sites, number of corpora lutea and of uterus weight. Determination of number of live and dead foetuses or embryos, determination of the sex and weight of each live foetus, and recording of number of runts, determination of individual placenta weights were performed. Examination was performed of all foetuses for external malformations, one part of the foetuses for visceral malformations (modified Wilson's method) and the rest of the foetuses for bone alterations (alizarin red S stained).

Results
Only the local skin irritation on the treated area was more common and more severe in females treated with the active substance than in control animals treated with vehicle only (statistically significant). No other effects were seen in this study on any of the parameters studied.

UK RMS Conclusion
Tebuconazole showed no developmental toxicity in this dermal "limit test" in rats using only 2 dose groups – controls and (high) dose (1000 mg/kg bw/day) treated animals. The daily administration of the test substance to the skin of rats on days 6-15 of gestation resulted in skin irritation in 9 treated animals while only 4 control animals had slight skin irritation. Under the conditions studied the dermal NOAEL for systemic maternal toxicity and developmental was 1000 mg/kg bw/day. These are the same NOAEL values agreed during the first review of tebuconazole.

B.6.6.2.1.3b Discussion and conclusion by DK-RMS:	DK RMS agrees with this conclusion and notes that a dermal application in general is expected to result in a lower systemic concentrations of the active compound than after oral dosing, due to ADME differences related to the exposure route.

B.6.6.2.1.4. Summary of rat developmental studies

The potential for tebuconazole to adversely affect development in the rat was investigated in seven studies; two standard guideline oral developmental toxicity studies, one new investigative (not to OECD test guideline) maternal toxicity study, two oral developmental neurotoxicity studies (not to OECD test guideline) and two studies via the dermal route (conducted to OECD test guidelines). In addition some studies from the open literature are relevant for the overall assessment of reproductive toxicity of tebuconazole. They are summarised in section B.6.6.3 and their results are included in the overall evaluation of reproductive toxicity, in section B.6.6.4.

Based on the regulatory studies summaried here, the UK RMS has provided the following summary

In the first oral study, developmental toxicity (increased incidence of total external malformations and microphthalmia, post-implantation loss and decreased foetal weight) was seen at the top dose of 100 mg/kg bw/d in the presence of significant maternal toxicity (significant reduction in body weight gain). No developmental toxicity was seen at 30 mg/kg bw/d at which there was still some maternal toxicity (reduced body weight gain). Based on these findings, a NOAEL of 30 mg/kg bw/d was identified for developmental toxicity and a NOAEL of 10 mg/kg bw/d was identified for maternal toxicity.

In the second oral study, similar effects on development (embryolethality, decreased foetal weight, reduced ossification and increased incidences of skeletal anomalies) were observed at the top dose of 120 mg/kg bw/d in the presence of significant maternal toxicity (reduced body weight gain, decreased food consumption and increased liver weight). No developmental toxicity occurred at 60 mg/kg bw/d at which there was still some maternal toxicity. Based on these findings, a NOAEL of 60 mg/kg bw/d was identified for developmental toxicity and a NOAEL of 30 mg/kg bw/d was identified for maternal toxicity.

A third study investigating maternal toxicity in more detail showed that the dose of 120 mg/kg bw/d caused severe maternal effects, including reduced body weight gain, decreased food consumption, clinical signs of toxicity, liver and adrenal toxicity.

In a regulatory dietary DNT study, developmental toxicity (pup mortality, reduced number of live born, reduced viability index, delayed vaginal patency, reduced body weight gain, reduced brain weight and decreased cerebellum thickness) was observed at the top dose of 65 - 125 mg/kg bw/d in the presence of significant maternal toxicity (mortality, reduced body weight gain and food consumption and prolonged gestation). Based on these findings, a NOAEL of 22 - 41 mg/kg bw/d was identified for both developmental and maternal toxicity from this study.

A published DNT study (B.6.6.2.1.2/01) is considered unreliable by the UK RMS.

In the two dermal developmental toxicity studies, there was no maternal or developmental toxicity up to the top dose of 1000 mg/kg bw/d.

Overall, developmental toxicity (pup mortality, reduced number of live born) was seen in rats from approximately 65 mg/kg bw/d (DNT study), increasing in severity (skeletal anomalies and increased incidence of total external malformations and microphthalmia) at around 100 - 120 mg/kg/bw/d. The overall NOAEL for developmental toxicity in rats was 30 mg/kg bw/d. The observed developmental toxicity was always associated with significant maternal toxicity and it is possible that some of these developmental effects were the secondary unspecific consequence of maternal toxicity. An overall NOAEL of 10 mg/kg bw/d was identified for maternal toxicity in rats.

B.6.6.2.1.4 Discussion and conclusion by DK-RMS:	DK RMS agrees with the summary of the results, but not that the B.6.6.2.1.2/01 study is unreliable. The DK-RMS also notes that several studies which are relevant for this assessment are found in the open literature. A discussion related to developmental and maternal toxicity for all relevant rats studies (both regulatory and published) is provided in section 6.6.4. Furhtermore the DK RMS does not agree with the following argumentation presented above: "*The observed developmental toxicity was always associated with significant maternal toxicity and it is possible that some of these developmental effects were the secondary unspecific consequence of maternal toxicity.*"

	DK RMS notes that in general, reductions in maternal body weight gain may be related to reduced food intake during early days of pregnancy and/or to specific toxicity to uterine and fetal growth. Therefore, on a case-by-case basis a thorough evaluation of relationships between maternal weight gain changes and fetal growth and survivial needs to be carried out. To aid this evaluation, tables comparing changes in maternal weight gains with changes in litter weights is presented for several rat studies exposed by gavage (see above). In these rat studies, reductions in maternal body weight gain in high dose groups were largest (in percent of control) in the first days of exposure. At termination of the studies, reduced fetal weights and reduced litter sizes caused reduced total litter weights, which could partly or fully explain the differences in maternal body weight gain during pregnancy. DK RMS concludes that it has not been demonstrated that the developmental effects are secondary to marked maternal systemic toxicity. In contrast, effects on mothers are likely related to specific modes of action causing developmental toxicity.

B.6.6.2.2. ***Rabbits***

Four developmental toxicity studies by oral administration in the rabbit were described in the original DAR (2006) (B.6.6.2.2.1/01; B.6.6.2.2.1/02; B.6.6.2.2.1/03 and B.6.6.2.2.1/04). Two different strains of rabbit were used - Himalayan CHBB:HM rabbits (B.6.6.2.2.1/01) and Chinchilla rabbits (B.6.6.2.2.1/02; B.6.6.2.2.1/03 and B.6.6.2.2.1/04). The study B.6.6.2.2.1/04 served as an investigative study of maternal toxicity and NOAELs were not derived. No new developmental toxicity studies in the rabbit were submitted.

B.6.6.2.2.1. **Developmental toxicity in rabbits after oral exposure**

a)

Previous evaluation	In DAR (2006) for original approval

Study ID	B.6.6.2.2.1/01
Study title	HWG 1608 proposed common name ethyltrianol – Study for embryotoxic effects on rabbits after oral administration
Matrix ID	23
Study dates	April to May 1984
Test substance	(HWG 1608) Tebuconazole
Purity (%) **Batch no.**	93.4 16007/83
Test animals	Mated female Himalayan CHBB:HM
Groups	15/dose
Dose	0, 3, 10, or 30 mg/kg on day 6-18 after mating (until mating had been observed (day zero of gestation))
Route	Oral by gavage
Vehicle	0.5% aqueous Cremophor emulsion. Dosing volume 5 mL/kg bw
GLP	Yes
Guideline	Not specified; comparable to OECD 414 (1981), valid at that time. The OECD guideline 414 has been revised in 2001 with extension of the dosing period to cover more than the organogenesis period and a few minor changes.
Deviation	Deviations from the version of the OECD guideline valid at the time of testing: food consumption was not recorded, corpora lutea were not counted, and foetuses were very small compared to other studies. The following deviations from the OECD-Guideline 414 (2001) occurred: - Bodyweight not reported in three-day intervals (for individual animals only weight gain during pregnancy and during treatment given).

	- Food consumption was not recorded. - Dosing was performed during the period of organogenesis only. - Reporting is not sufficient – no raw data presentation. No corpora lutea data were given. Numbers of pre-implantation losses were not given. No individual body weights of foetuses. Uterus weight not reported.
Acceptable	Acceptable as supplementary information only, due to the poor reporting, reduced data base being available for inspection, doses tested being low (not tested up to maternal toxicity) and increased number of losses not being commented.
NOAEL	Maternal toxicity NOAEL: 10 mg/kg bw/day. Developmental toxicity NOAEL: 10 mg/kg bw/day.
Effects at the LOAEL	Maternal toxicity: decreased body weight gain during dosing at 30 mg/kg bw/day. Developmental toxicity: increased resorptions at 30 mg/kg bw/day
DK RMS disagree	NOAEL for maternal toxicity: 30 mg/kg/bw/d. Only a nominal decrease in maternal body weight gain was observed during dosing in the 30 mg/kg bw/day group, it was non-significant and these high dose dams actually gained more weight from day 0-29 than control dams (also non-significant). This is in agreement with the maternal NOAEL for the last review.

Methods

Groups of 15 mated female Himalayan CHBB:HM were given by gavage daily doses of 0, 3, 10, or 30 mg/kg pure HWG 1608 on days 6 to 18 of gestation. The rabbits were inspected daily for deaths and clinical signs and were weighed daily (dosing was related to the recent body weight). On day 29 of gestation the animals were killed and the uteri were removed by Caesarean section. The following determinations were made: Number of implantation, number of live or dead foetuses or embryos, sex of all live foetuses, litter weight and mean foetus weight per litter and total and mean placenta weight. The foetuses were inspected in detail for external malformation, and the skull were examined for visceral malformations (stained by a modified Wilson's technique) and finally the foetuses were exenterated for appraisal of abdominal and thoracic organs and subsequently cleared with diluted potassium hydroxide solution and stained for appraisal of the bone system (with Alizarin Red S).

Results

Maternal toxicity

No changes in the dams' appearance and behaviour which might be seen as results of the treatment were noted in the daily inspections. One dam of the control group died prior to sacrifice. There was reduced body weight gain (-26 % change compared to control) in dams of the highest dose group (30 mg/kg bw/day) during dosing.

Table 6.6-35. Maternal toxicity

Parameter		Control data	Dose (mg/kg bw/day)					
			3		10		30	
		Study	*(%)[a]*		*(%)[a]*		*(%)[a]*	
Number of dams examined		13	14		14		15	
Mortality of dams %		7.4	0		0		0	
Abortions		0	0		0		0	
Body weight gain [g]	day 6-18 (dosing period)	80.0	82.6	*(3)*	74.9 (94),	*(-6)*	**59.5**	***(-26)***
	day 0-29	260.2	272.4	*(5)*	323.6	*(24)*	300.4	*(15)*
Pregnancies %		100	100		100		100	
Necropsy findings in dams dead before end of test		+						

(%)[a] % change compared to control

Caesarean section data

At 30 mg/kg bw/day a statistically significant increase in resorptions per dam occurred (300 % change compared to control), however this value was within the HCD range (0.2 – 2.6) shown. There was also an increase in post-implantation losses at the top dose (265 % change compared to control), however this value was within the HCD range (2.6 – 38.8) shown. All other reproductive parameters, including number of foetuses/litter, the incidence of dead foetuses, placenta weight and foetal sex ratio, were unaffected by treatment up to and including 30 mg/kg bw/day.

Table 6.6-36. Intrauterine development

Parameter	Control data		Dose (mg/kg bw/day)					
	Historical§	Study	3		10		30	
Implantations / dam		6.4	7.1		7.9		7.1	
Resorptions / dam *(%)[a]*	**0.2 – 2.6**	0.2 -	0.6 *(200)*		0.5 *(150)*		**0.8*** ***(300)***	
Early resorptions (mean per dam)		0	0.3		0.2		0.3	
Late resorptions (mean per dam)		0.2	0.3		0.3		0.5	
Total number of foetuses		80	91		103		94	
Post-implantation loss [%] *(%)[a]*	2.6 – 38.8	3.1 -	8.5 *(174)*		6.3 *(103)*		**11.3** ***(265)***	
Total number of litters		13	14		14		15	
Foetuses / litter		6.2	6.5		7.4		6.3	
Dead foetuses / litter		0	0		0		0	
Foetus weight, mean [g]		39.28	40.22		39.32		40.85	
Placenta weight, mean [g]		4.57	4.69		4.37		4.59	
Foetal sex ratio [m/f]		2.9 3.2	2.9	3.6	3.5	3.9	3.3	2.9

*/** significantly different from study controls *($p \leq 0.05$ / $p \leq 0.01$)*

§ Resorption: Historical control data (HCD) range from 1982 – 1990 (36 studies, 481 litters, 2870 foetuses), from performing laboratory. Early/late resorption: HCD from performing laboratory only available from 1988 – 1993 (12 studies, 173 litters, 1056 foetuses).

(%)[a] percent change compared to study control

External, visceral and skeletal examination of foetuses

No increased incidences of malformations or other indications of developmental toxicity were observed up to and including 30 mg/kg bw/day.

Table 6.6-37. Foetal findings

Parameter		Control data		Low dose 3 mg/kg		Medium dose 10 mg/kg		High dose 30 mg/kg	
		Historical §	Study		*(%)[a]*		*(%)[a]*		*(%)[a]*
Number foetuses			80	91	*(14)*	103	*(29)*	94	*(18)*
Number litters			13	14	*(8)*	14	*(8)*	15	*(15)*
External									
Limb (fore- or hind-) hyperflexion (arthrogryposis) (%)	F	0-4.3	0.0	1.1		1.0		0.0	
	L		0.0	7.1		7.1		0.0	

§ Historical control data (HCD) range from 1982 – 1993 (42 studies, 557 litters, 3439 foetuses), from performing laboratory.

F: % foetuses, L: % litter

(%)[a] percent change compared to study control

DK RMS Table with body weight calculations

ID	Reference	Species	BW discussion

23	Study rep, 1985 (Renhof 1985b)	Rabbit	- Doses: 0, 3, 10, 30 mg/kg - GD 6-18 - **30 mg/kg:** Dam difference in weight gain exp period: 80 g (control) - 59.5 g (30 mg/kg) = 20.5 g lower than control Dam difference in weight gain during pregnancy period: 260.2 g (con) - 300.4 g (30 mg/kg) = + 40 g heavier than control Litter weight 6.2 * 39.28 g (con) - 6.3 * 40.85 = + 13.85 g heavier than control - *No observed effects were seen on body weight at the end of study.*

UK-RMS Conclusion

Under the conditions of this non-guideline teratogenicity study in Himalayan rabbits, a slight increase in resorptions was seen at the top dose of 30 mg/kg bw/day at which some maternal toxicity occurred (reduction in body weight gain). It is most likely the resorptions were the secondary unspecific consequence of the maternal toxicity observed. Overall, a NOAEL of 10 mg/kg bw/day can be identified from this limited study for maternal and developmental toxicity. It should be noted that the maternal NOAEL has been lowered from the top dose of 30 mg/kg bw/day to 10 mg/kg bw/day; this NOAEL is based on reduced body weight gain in dams of the highest dose group (30 mg/kg bw/day – the next highest dose). Thus, this NOAEL differs from that agreed during the first review of tebuconazole. The developmental toxicity NOAEL is unchanged from that agreed in the DAR (2006).

B.6.6.2.2.1a Discussion and conclusion by DK-RMS:	DK RMS notes that while a nominal decrease in maternal body weight gain was observed during dosing in the 30 mg/kg bw/day group, it was non-significant and these high dose dams actually gained more weight from day 0-29 than control dams (also non-significant). The DK-RMS therefore does not find the maternal effects seen at 30 mg/kg as adverse or toxicologically relevant, and finds that the maternal NOAEL should be 30 mg/kg bw/day and not 10 mg/kg bw/day, as proposed by the UK-RMS. The high-dose foetuses were not small compared to control offspring, but an increase in resorptions and post-implantation loss was observed. Due to the slight maternal toxicity, these effects were in the opinion of the DK RMS not very likely secondary unspecific consequences of maternal toxicity, but rather caused specifically by prenatal exposure to tebuconazol. The findings were however inside the given HCD range (0.76 - 1.76 %, n = 44)) in the study report from 3 “comparable studies” (no mean and SD were presented), but the validity of these could not be fully elucidated, test species and strain was not specified. Other HCD mentioned under the tables as coming from performing lab (1982-1990) could not be verified as they seem to not have been submitted to DK-RMS. The conclusion is supported by similar results from studies performed in mice and rats. ***This study is only supportive due to reporting deficiencies etc.***

b)

Previous evaluation	In DAR (2006) for original approval

Study ID	B.6.6.2.2.1/02
Study title	Embryotoxicity study (including teratogenicity) with HWG 1608 technical in the rabbit
Matrix ID	24
Study dates	January 5 1987 – February 13 1987
Test substance	(HWG 1608) Tebuconazole technical
Purity (%) **Batch no.**	98.2 16002/85
Test animals	Mated female Chinchilla rabbits, CHIN, hybrids, SPF quality (until mating observed)
Groups	16/dose
Dose	0, 10, 30, or 100 mg/kg bw/day on day 6-18 post mating
Route	Oral by gavage
Vehicle	0.5 % aqueous Cremophor EL solution. Dosing volume was 4 mL/kg bw

GLP	Yes
Guideline	OECD Guidelines for the Testing of Chemicals, 414 "Teratogenicity" (1981). The OECD guideline 414 has been revised in 2001 with extension of the dosing period to cover more than the organogenesis period and a few minor changes
Deviation	No deviations in relation to the version of the OECD guideline 414 (1981) (valid at the time of testing). The following deviations from the OECD-Guideline 414 (2001) occurred: - Dosing was performed during the period of organogenesis only. - Food consumption recorded in 4 or 5-day intervals instead of three-day intervals. - Only 15 dams with implantation sites per group instead of at least 16. - double staining not performed The deviations are not found to totally compromise the results.
Acceptable	Acceptable, with restrictions.
NOAEL	Maternal and developmental toxicity: 30 mg/kg bw/day.
Effects at the LOAEL	Maternal toxicity: decreased food consumption and reduced body weight gain at 100 mg/kg bw/day. Developmental toxicity: increased post-implantation losses and an increase in malformations and anomalies at 100 mg/kg bw/day.

Methods
In accordance with OECD Guideline for the Testing of Chemicals No. 414 (1981) (Teratogenicity) (and GLP) four groups of each 16 mated female Chinchilla rabbits (CHIN hybrids, SPF quality) were given by gavage daily doses of 98.2 % pure tebuconazole technical at, 0, 10, 30 or 100 mg/kg bw suspended in 0.5 % aqueous Cremophor EL solution on days 6 - 18 of gestation. The animals were observed daily for mortality, clinical signs and behaviour. The animals were weighed daily and the test substance was dosed according to the actual body weight. Food consumption was recorded on days 6, 11, 15, 19, 24 and 28 post coitum.

The dams were killed (by cervical dislocation) on day 28 of gestation and foetuses removed by Caesarean section and the livers of all dams were weighed and fixed in formaldehyde solution. Necropsy included: gross macroscopic examination of all internal organs, with emphasis on the uterus, uterine contents, position of foetuses in the uteri and number of corpora lutea. The foetuses were removed from the uterus, weighed, examined for gross external abnormalities and prepared for internal examinations. Foetuses were dissected and body cavities (thorax, abdomen, pelvis) and all organs were examined and abnormalities recorded. Skin was removed and the crania of all foetuses were examined for ossification. The heads were serially sectioned and examined. The skeletons were examined (after clearing in potassium hydroxide solution and staining with Alizarin Red S). All abnormalities and variations were recorded. Abnormal foetuses were photographed.

The uteri and uterine contents of all pregnant females were weighed (non-pregnant uteri were placed in an ammonium sulphide solution to accentuate possible haemorrhagic areas of implantation sites).

Results
Maternal toxicity
No mortalities or clinical signs related to dosing were observed in any dose or control group. Maternal toxicity occurred at 100 mg/kg bw/day. At this dose, feed intakes were decreased (≥ 10 % change compared to control during treatment) and body weight loss occurred from day 6 to 8 p.c. which was not compensated during the remaining gestation period. Overall body weight gain during the treatment period was also decreased (statistically significant difference and -38 % change compared to control during treatment) (Table 6.6-37.).

Necropsy observations
None of the necropsy findings in the dams were considered to be related to dosing with tebuconazole. No treatment-related or statistically significant differences were noted between the liver weights and liver/body weight ratios of the dose groups and the vehicle control group (Table 6.6-38.).

Table 6.6-38. Maternal toxicity

Parameter		Control data	Low dose 10 mg/kg		Medium dose 30 mg/kg		High dose 100 mg/kg	
		Study		*(%)[a]*		*(%)[a]*		*(%)[a]*
Number of dams examined		15	14		15		14	
Clinical findings[1)]		-	-		-		-	
Mortality of dams %		0	0		0		0[2)]	
Abortions		0	0		0		0	
Body weight gain [g]	GD 0-6	205	235	*(+15)*	242	*(+18)*	205	*(±0)*
	GD 6-8	63	29	*(-54)*	21	*(-67)*	-34	*(-154)*
	GD 6-11	138	93	*(-33)*	95	*(-31)*	**28**	***(-80)***
	GD 11-15	87	71	*(-18)*	99	*(+14)*	78	*(-10)*
	GD 15-19	94	98	*(+4)*	108	*(+15)*	92	*(-2)*
	GD 6-28	464	442	*(-5)*	453	*(-2)*	**341**	***(-27)***
during treatment	GD 6-19	319	262	*(-18)*	302	*(-5)*	**198***	***(-38)***
Food consumption [g]	GD 0-6	195	199	*(+2)*	201	*(+3)*	202	*(+4)*
	GD 6-11	210	201	*(-4)*	199	*(-5)*	**179**	***(-15)***
	GD 11-15	197	194	*(-2)*	202	*(+3)*	**177**	***(-10)***
	GD 15-19	214	201	*(-6)*	210	*(-2)*	**192**	***(-10)***
during treatment	GD 6-19	207	199	*(-4)*	203	*(-2)*	**182**	***(-12)***
Pregnancies %[3)]		100	87.5		100		93.3	
Necropsy findings in dams dead before end of test							damaged lung	

GD: gestation day; [a]: % change compared to control
[1)] during application of test substance [2)] 1 dam intubation error
[3)] note that animals were dosed from day 6 of gestation
* Body weight gain day 6 – 18 in the high dose, 100 mg/kg, was identified as significantly different from study controls *($p \leq 0.05$),* with a value of 189.

Table 6.6-39. Organ weights

	Dose (mg/kg bw/day)			
	0	**10**	**30**	**100**
Females				
Number of individuals	16	16	16	15
Body weight (g) mean (± s.d.)	3470 *(±360)*	3464 *(±204)*	3571 *(±294)*	3598 *(±327)*
Liver weight (g) mean (± s.d.)	81.88 *(±14.01)*	74.44 *(±9.13)*	76.61 *(±9.99)*	84.62 *(±12.40)*
Liver (%) mean (± s.d.)	2.36 *(±0.35)*	2.15 *(±0.23)*	2.14 *(±0.19)*	2.35 *(±0.24)*

s.d. standard deviation

Developmental toxicity

There was no effect on intrauterine development up to and including 30 mg/kg bw/day. The dose of 100 mg/kg bw/day resulted in a markedly increased resorption rate (both early and late, > 10 % change compared to control), an increase in post-implantation loss (> 10 % compared to control) (Table 6.6-39.), and a slight reduction in the number of liver foetuses (> 10 % compared to control for group and % of implantations). The applicant provided HCD with the aim of demonstrating that early and late resorptions, and pre-implantation loss is within the HCD range, however the UK-RMS notes that the date range for HCD is 1989 – 1995, not entirely within ± 5 years of the current study date 1987.

DK-RMS agrees that the historic controls is outside the acceptable range and notes that results should therefore only be compared to concurrent controls.

A marginal decreased foetal body weight (6 % change compared to control) (Table 6.6-39.) was seen, which correlated with slightly retarded ossification (Table 6.6-40). In addition, an increased incidence of external malformations (total incidence of 33.3 at 100 mg/kg bw/day compared to 0 in control) (hemimelia, agenesis of claws, malrotation of hind limbs, enlarged fontanelle or cleft palate) occurred at 100 mg/kg bw/day.

Table 6.6-40. Intrauterine development

Parameter	Control data		Dose (mg/kg bw/day)		
	Historical[$]	Study	10	30	100
Number of dams		15	14	15	14
Corpora lutea / dam	1.8 – 12.6	8.5	8.9	9.3	9.4
Implantations / dam	7.5 – 12.1	8.1	8.3	8.9	8.9
Post-implantation loss (Resorptions)					
% of implantations	2.3 – 22.1	8.3	2.6	8.3	**27.4[1]**
(%)[a]	-	-	*(-69)*	*(±0)*	***(230)***
/dam	0.2 – 2.5	0.7	0.2	0.7	**2.4**
(%)[a]	-	-	*(-71)*	*(±0)*	***(243)***
Embryonic (early) resorptions					
/group	-	2	2	5	**12**
% of implantations	-	1.7	1.7	3.8	**9.7**
/dam	0.0 – 1.2	0.1	0.1	0.3	**0.9**
(%)[a]	-	-	*(±0)*	*(200)*	***(800)***
Foetal (late) resorptions					
/group	-	8	1	6	**22**
% of implantations	-	6.6	0.9	4.5	**17.7**
/dam	0.1 – 1.8	0.5	0.1	0.4	**1.6**
(%)[a]	-	-	*(-80)*	*(-40)*	***(220)***
Pre-implantation loss					
[%]	0.0 – 9.0	5.5	7.2	5.0	6.1
/dam	-	0.5	0.6	0.5	0.6
Total number of foetuses					
/group	3225	111	113	122	**90**
(%)[a]	-	-	*(2)*	*(10)*	***(-19)***
/dam	-	7.4	8.1	8.1	6.4
Live, % of implantations	-	91.7	97.4	91.7	**72.6**
(%)[a]	-	-	*(6)*	*(±0)*	***(-21)***
Total number of litters	346	15	14	15	14
Foetuses / litter	6.9 – 11.2	7.4	8.1	8.1	6.4
Dead foetuses / litter ratio	0	0	0	0	0
Foetus weight [g, mean] *(%)[a]*	29.2 – 34.4	35.1	33.5 *(-5)*	35.0 *(±0)*	33.0 *(-6)*
Foetal sex ratio (males/females)	-	59/52	57/56	62/60	46/44

[1]: Only the % of foetal resorptions (17.7 % of implantations) was significantly increased over controls (6.6 % of implantations)
[$]: Historical control data range from 1989 – 1995 (18 studies, 346 litters, 3225 foetuses) (data from the same laboratory, on Chinchilla rabbits, SPF quality, not entirely within ± 5 years of the current study date 1987).
(%)[a]: % change compared to control

Table 6.6-41. Foetal findings

Parameter		Control data		Dose (mg/kg bw/day)		
		Historical[$]	Study	10	30	100
Number foetuses		-	111	113	122	90
Number litters		-	15	14	15	14
External malformations						
Foetuses affected			0	0	0	**8**
Litters affected			0	0	0	**5**
Total incidences	F	0.0 – 4.5	0	0	0	**8.9**
	L	*Could not be calculated*	0	0	0	**33.3**
Cleft Palate	F	0.0 – 0.7	0	0	0	**1.1**
	L	0.0 – 6.7	0	0	0	**6.7**
Malrotation of hind limb	F	0.0 – 0.6	0	0	0	**1.1**
	L	0.0 – 6.3	0	0	0	**6.7**
Hemimelia (peromelia)	F	*no HCD range*	0	0	0	**5.6**
	L	*no HCD range*	0	0	0	**26.7**
Agenesis of claws	F	*no HCD range*	0	0	0	**1.1**
	L	*no HCD range*	0	0	0	**6.7**
Skeletal findings						
Foetuses affected			0	1	2[a]	**6[b]**
Litters affected			0	1	2	4
Total incidence	F	*no HCD range*	0	0.9	1.6	**6.7**
	L	*no HCD range*	0	6.7	12.5	**26.7**
Visceral findings						
Hydrocephalus internus	F	0.0 – 0.6	0	0	0	**1.1**
	L	0.0 – 6.7	0	0	0	**6.7**

F: % foetuses, L: % litter
[$]: Historical control data range from 1989 – 1995 (18 studies, 346 litters, 3225 foetuses) (data from the same laboratory, on Chinchilla rabbits SPF quality, not entirely within ± 5 years of the current study date 1987)
[a]: one finding of abnormally ossified and fused sternebrae nos. 2 to 4
[b]: one finding of abnormally ossified and fused sternebrae nos. 4 to 5 and one finding of broadened distal portion of rib no. 7 (one sided).

DK RMS Table with body weight calculations

ID	Reference	Species	BW discussion

24	Study rep, 1988 B.6.6.2.2.1/02	Rabbit	- Doses: 0, 10, 30, 100 mg/kg - GD 6-18 **- 100 mg/kg:** Weight of treated litters (33g * 6.4 pups/litter = 211.2 g) compared to control litters (35.1 g * 7.4 pups/litter = 259.7 g) resulted in a difference of -48 g. This cannot account for all of the reduced weight gain of dams seen at GD 6-19 and GD 6-28. (123 g difference to control on GD 6-28 and 121 g difference to control GD 6- 19) *The lower litter weight cannot explain the reduced maternal weight. Food consumption seems to be reduced during exposure and this was not caused by poor palatability since exposure was done via oral gavage.*

UK RMS Conclusion
In this guideline oral developmental toxicity study in Chinchilla rabbits, developmental toxicity (markedly increased resorptions, slightly decreased numbers of live foetuses, marginally decreased foetal weight and slightly increased incidence of a number of external malformations, including cleft palate, malrotation of hind limb, hemimelia and agenesis of claws) was seen at the top dose of 100 mg/kg bw/day, at which maternal toxicity (reduced feed intake and body weight loss) occurred. On this basis, the NOAEL for maternal toxicity and developmental toxicity was 30 mg/kg bw/day. These are the same NOAEL values agreed during the first review of tebuconazole.

B.6.6.2.2.1b Discussion and conclusion by DK-RMS:	DK RMS notes that there was no actual body weight loss at the time of necropsy, indicating that the maternal systemic toxicity in the high dose group was not marked. The observed decrease in maternal body weight gain during dosing can not be explained by a lower number of foetuses with lower body weights. Updated HCD data: B.6.6.2.2.1/03

c)

Previous evaluation	In DAR (2006) for original approval

Study ID	B.6.6.2.2.1/03
Study title	Combined report of embryotoxicity study (including teratogenicity) and supplementary investigations on the maternal toxicity of HWG 1608 technical (c.n. Tebuconazole) in pregnant rabbits
Matrix ID	25
Study dates	March – April 1992
Test substance	(HWG 1608) Tebuconazole technical
Purity (%) **Batch no.**	96.8 or 96.3 for the main study 96.8 in the supplementary study 816196048
Test animals	Mated female Chinchilla rabbits (CHbb: CH, Hybrids, SPF quality) (mated until copulation had been observed)
Groups	16/dose in the main study and 5/dose in the supplementary study
Dose	0, 10, 30, or 100 mg/kg bw/day on day 6-18 post mating
Route	Oral by gavage
Vehicle	0.5 % Cremophor EL in bidistilled water. Total volume administered 4 mL/kg bw/day
GLP	Yes
Guideline	OECD Guideline for the Testing of Chemicals No. 414 (“Teratogenicity”), May 1981, and EPA, Pesticide Assessment Guidelines 83-3 (“Teratogenicity Study) revised edition,

	November 1984. Both the EPA and the OECD guidelines have been revised (OECD guideline 414 in 2001) with extension of the dosing period to cover more than the organogenesis period and a few minor changes
Deviation	No deviations in the main study with respect to the guidelines referred to. The extension with a supplementary (smaller) study intended to yield further parameters (e.g. clinical chemistry parameters and organs weights) on the dams was too small to comply with a guideline. Compared to the recent versions of the guidelines a number of deviations have been identified but these do not compromise the results as reported. The following deviations from the OECD-Guideline 414 (2001) occurred: - Dosing was performed during the period of organogenesis only. - Food consumption recorded in 4 or 5-day intervals instead of three-day intervals. - Not at least 16 dams with implantation sites in each group. The extension with a supplementary (smaller) study intended to yield further parameters (e.g. clinical chemistry parameters and organs weights) on the dams. The supplementary study is acceptable as supplementary information due to the low number of animals tested. The deviations identified do not compromise the results as reported.
Acceptable	The main study is acceptable The supplementary study is only acceptable as supplementary information due to the low number of animals tested (and even lower number of pregnancies)
NOAEL	UK-RMS:Maternal toxicity NOAEL: 30 mg/kg bw/day. Developmental toxicity NOAEL: 30 mg/kg bw/day (increased from the value of 10 mg/kg bw/day agreed in the previous review). DK-RMS: Developmental toxicity NOAEL: 10 mg/kg bw/day based upon dose-related increased incidence of malformations at the two highest doses (NOAEL as agreed in the previous review).
Effects at the LOAEL	UK-RMS:Maternal toxicity: Decreased food consumption and reduced body weight gain at the ~~next~~ highest dose 100 mg/kg bw/day (the top dose). Developmental toxicity: Increased post-implantation loss, reduced foetal weight and increased incidence of malformations at the next highest dose 100 mg/kg bw/day (the top dose). DK-RMS: Developmental toxicity LOAEL: 30 mg/kg bw/day based upon treatment-related malformations at the two highest doses (NOAEL as agreed in the previous review).

Methods
In accordance with the OECD Guideline for the Testing of Chemicals No. 414 ("Teratogenicity"), May 1981, and the EPA, Pesticide Assessment Guidelines 83-3 ("Teratogenicity Study") revised edition, November 1984, groups of 16 (main study) or 5 (supplementary study) mated Chinchilla rabbits (CHbb: CH, Hybrids, SPF quality) were given by gavage once daily on day 6 through to day 18 of gestation doses of tebuconazole. The test substance (96.30 - 96.80 % pure) was given suspended in 0.5 % Cremophor EL solution in doses of 0, 10, 30, or 100 mg/kg bw. The animals were inspected for mortality, clinical signs and changes in appearance or behaviour at least twice daily. Body weight was recorded daily and food consumption at regular intervals.

Main study: The dams were necropsied on day 28 of gestation, and the gravid uteri were removed by caesarean section and weighed before opening. The following data were recorded in pregnant dams: Number of corpora lutea, number of implantation sites, litter size, position, weight and sex of each live foetus, and number of dead foetuses. The foetuses were examined for external abnormalities, and visceral and skeletal abnormalities and skeletal retardations.

Supplementary study: Blood specimens were taken from the marginal ear vein on days 6, 12 and 19 post-coitum and subjected to full haematological and clinical chemistry analysis. The dams were sacrificed just after the last blood specimens had been taken, and the gravid uteri were removed by caesarean operation and weighed. All reproduction parameters were recorded (as above). The adrenals, the kidneys, the liver and the spleen from all gravid dams were weighed separately and prepared for histological examinations. Two portions of each 10 grams were before the preparation taken from each liver for determination of the cytochrome P-450, the N-demethylase and the O-demethylase activities and the triglyceride content.

Results

Maternal toxicity

Main study

In the main study, no treatment-related deaths or symptoms were evident. At 10 and 30 mg/kg bw/day, food consumption was not influenced by treatment. At 100 mg/kg bw/day feed intakes were decreased (- 17 % compared to control during treatment (GD 6 – 19)). Body weight gain of dams was not influenced by treatment at 10 mg/kg bw/day, however at 30 mg/kg bw/day this was increased in dams (> 10 % change compared to control during treatment) and at 100 mg/kg bw/day this was markedly decreased (59 % change compared to control during treatment). At the top dose body weight loss occurred from day 6 - 11 p.c. (- 51 g) which was not compensated during the remaining gestation period. Overall body weight gain during the treatment period was also markedly decreased in the 100 mg/kg bw/day group (Table 6.6-41.).

Table 6.6-42. Maternal effects (main study)

Parameter		Control data		Dose (mg/kg bw/day)					
				10		30		100	
		Historical[$]	Study		*(%)[a]*		*(%)[a]*		*(%)[a]*
Number of dams examined		272	16	15		14		14	
Clinical findings during application of test substance			-	-		-		-	
Mortality of dams %			0	6.3 incidental		0		0	
Abortions			0	0		0		0	
Body weight gain [g]	GD 0-6		215	171	*(-20)*	198	*(-8)*	207	*(-4)*
	GD 6-11		55	43	*(-22)*	62	*(+13)*	-51	*(-193)*
	GD 11-15		37	33	*(-11)*	43	*(+16)*	48	*(+30)*
	GD 15-19		43	41	*(-5)*	52	*(+21)*	59	*(+37)*
	GD 6-28		259	256	*(-1)*	267	*(+3)*	187	*(-28)*
during treatment	GD 6-19		135	117	*(-13)*	**157**	***(+16)***	**56**	***(-59)***
Food consumption [g]	GD 0-6		226	206	*(-9)*	221	*(-2)*	218	*(-4)*
	GD 6-11		229	209	*(-9)*	223	*(-3)*	162	*(-29)*
	GD 11-15		201	181	*(-10)*	198	*(-1)*	180	*(-10)*
	GD 15-19		183	176	*(-4)*	202	*(+10)*	173	*(-5)*
during treatment	GD 6-19		206	190	*(-8)*	209	*(+1)*	**171**	***(-17)***
Pregnancies %		93.4	100	93.8		100		93.8	
Necropsy findings in dams dead before end of test				Death incidental					

GD: Gestation day

[$]: Historical control data range from 1989 – 1995 (18 studies, 346 litters, 3225 foetuses) (data from the same laboratory, on Chinchilla rabbits, SPF quality, within ± 5 years of the current study date 1992).

[a]: % change compared to control

DK RMS Table with body weight calculations

ID	Reference	Species	BW discussion

25	Study rep, B.6.6.2.2.1/03	Rabbit	- Doses: 0, 10, 30, 100 mg/kg (included a main study and a supplementary study. The data from the supplementary study comes from 3-5 dams/group and data is therefore not included) - GD 6-19 **- 100 mg/kg:** Dam difference in weight gain exp period: 135g (control) – 56g (100 mg/kg) = 79 g lower than control Dam difference in weight gain pregnancy period: 259g (control) – 187g (100 mg/kg) = 72 g lower than control Litter weight 8.8 * 31.5 (control) - 8.5 * 30 (100mg/kg) = 22.2 g lower than control *- The lower litter weight cannot explain the reduced maternal weight . Food consumption was reduced during exposure (not statistically significant, but 17% lower in 100 mg/kg group)*

Supplementary study

The supplementary study revealed reduced body weight gain (> 10 % change compared to control) and food consumption (< 10 % change compared to control) at the top dose (Table 6.6-42.). At 100 mg/kg bw/day a reduction in % pregnancies was also evident (however, it is noted that pregnancy was established before the start of treatment), at – 25 % change compared to control, a value outside of the HCD range (HCD from DAR, further information on HCD not available).

Histopathological evaluation of the livers in the supplementary study showed an increase in single cell necrosis in all treated animals compared to controls and one finding of necrosis at 30 mg/kg bw/day (Table 6.6-43.). The number of animals investigated was too low to permit definitive interpretation of these findings. In addition, no dose-response was observed in both incidence and severity of these liver histopathological findings. Although the liver is known to be the main target organ in the other species examined, a clear treatment-related effect (from 10 mg/kg bw/day upwards) cannot be determined from this limited study.

Table 6.6-43. Maternal effects (supplementary study)

Parameter		Control data		Dose (mg/kg bw/day)		
		Historical	Study	10	30	100
Number of dams examined		272	4	4	5	3
Clinical findings during application of test substance			-	-	-	-
Mortality of dams %			20 incidental	0	0	0
Abortions			0	0	0	0
Body weight gain (g)	GD 6-19		-112	76	46	**-146**
	(%)[a]		-			***(-30)***
Food consumption (g)	GD 6-19		105	171	144	**96**
	(%)[a]		-			***(-9)***
Pregnancies %		93.4	100	100	100	**75**
	(%)[a]		-			***(-25)***

(%)[a]: % change compared to control

Table 6.6-44. Necropsy findings (supplementary study)

Parameter	Dose (mg/kg bw/day)			
	0	10	30	100
No of animals	4	5	5	5

Parameter		Dose (mg/kg bw/day)			
		0	**10**	**30**	**100**
Liver					
Vacuolization	total affected	4	4	5	4
	mean severity	2.8	2.8	2.0	3.0
Single cell necrosis	total affected	1	5	5	5
	mean severity	1.0	1.0	1.2	1.0
Necrosis	total affected	-	-	1	-
	mean severity	-	-	1.0	-
Sinusoidal leucocyte.	total affected	-	-	3	2
	mean severity	-	-	1.0	1.5

Developmental toxicity

Main study

In the main study, the reproduction parameters affected at 100 mg/kg bw/day were: an increased mean post-implantation loss (24 % change compared to control, a value outside of the HCD range), an increased number of resorptions (28 % change compared to control, a value outside of the HCD range) and a decrease in mean foetus weight (statistically significant but < 10 % change compared to control) (Table 6.6-44). A clear dose-response relationship was not evident; effects seen could be secondary to maternal toxicity (body weight gain decrease at 100 mg/kg bw/day).

Table 6.6-45. Intrauterine development (main study)

Parameter	Control data		Dose (mg/kg bw/day)					
	Historical[$]	**Study**	**10**	*(%)[a]*	**30**	*(%)[a]*	**100**	*(%)[a]*
Corpora lutea / dam	7.8 – 12.6	11.9	12.3		11.4		11.9	
Implantations / dam	7.5 – 12.1	11.3	11.3		9.7		11.7	
Resorptions / dam	0.2 – 2.5	2.5	1.9	*(-24)*	2.6	*(4)*	**3.2**	***(28)***
Early resorptions (mean per dam)	0.0 – 1.2	1.1	0.7	*(-36)*	1.6	*(46)*	**1.9**	***(73)***
Late resorptions (mean per dam)	0.1 – 1.8	1.4	1.2		0.9		1.3	
Total number of foetuses	3225	141	142		109		119	
Pre-implantation loss [% corp lutea]	0.0 – 9.0	5.2	8.1		9.4		1.2*	
Post-implantation loss [% impl.]	2.3 – 22.1	22.1	16.5	*(-25)*	24.8	*(12)*	**27.4**	***(24)***
Total number of litters	346	16	15		14		14	
Foetuses / litter	6.9 – 11.2	8.8	9.5		7.8		8.5	
Dead foetuses / litter	0	0	0		0		0	
Foetus weight, mean [g] *(%)[a]*	29.2 – 34.4	31.5	32.0	*(+2)*	31.6	*(±0)*	30.0*	*(-5)*
Foetal sex ratio (males/females)		1.04	1.29		0.93		0.92	

*/** significantly different from study controls *($p \leq 0.05$ / $p \leq 0.01$)*

[$]: Historical control data range from 1989 – 1995 (18 studies, 346 litters, 3225 foetuses) (data from the same laboratory, on Chinchilla rabbits, SPF quality, within ± 5 years of the current study date 1992).

[a]: % change compared to control

The 30 and 100 mg/kg bw groups had a statistically significant increase in foetuses with abnormalities and malformations (Table B.6.6-45) compared to the concurrent control group and also to the historical control data available at that time (covering period 1989-1992). At 30 mg/kg bw/day, three foetuses with external malformations (one foetus with malpositioned hind legs, one foetus with arthrogryposis, one foetus with multiple malformations; two of these were runts) were observed. Abnormal findings at 100 mg/kg bw/day were noted in four foetuses (two runts; one with acephaly and multiple other malformations; two foetuses with meningocele in the area of the os parietale and both with other malformations) during external (3) and visceral (1) examinations. Additional findings were evident in two of the four 100 mg/kg bw/day foetuses during examination of the heads by Wilson technique and in two of the four foetuses during skeletal examinations for abnormal findings (Table B.6.6-46.). Slightly increased incidences of non- and incomplete ossification which correlated with statistically significant reductions of mean foetal body weight (individual basis) were also noted in this top dose group.

Table 6.6-46. Examination of foetuses (main study)

Parameter	Control data		Dose (mg/kg bw/day)		
	Historical (available at the time of the study covering period 1989-1992)	Study	10	30	100
External malformations[*1] [%]	1.2	0	1.4	**3.7***	**3.4***
Skeletal malformations[*2] [%]of the heads					0.8
Skeletal anomalies[*3] [%]	1.3	2.8	2.8	2.8	3.7
Visceral malformations[*4] [%]	0.8	0	0	0	3.3

[*1]: Control group: No abnormal findings. 10 mg/kg bw dose group: two runts. 30 mg/kg bw dose group: one foetus with malpositioned hind legs, one foetus with arthrogryposis, two foetuses were runts and one of these had multiple malformations. 100 mg/kg bw dose group: two runts one with acephaly and multiple other malformations, two foetuses with meningocele in the area of os parietale and both with other malformations.
[*2]: Two findings of indentation of skull (one with protrusion of brain).
[*3]: Control group: Four foetuses with missing vertebral bodies and all with other skeletal findings too. 10 mg/kg bw dose group: ribs fused or missing and vertebral bodies missing in four foetuses. 30 mg/kg bw dose group: ribs rudimentary or missing and vertebral bodies and arch missing in one foetus, toe bones missing in one foetus, and sternebrae nos. 1-4 asymmetrically ossified in one foetus. 100 mg/kg bw dose group: one foetus with thoracic vertebral body and arch no. 13 missing – ribs nos. 11 and 12 rudimentary and fuse, one foetus with acephaly and many other skeletal malformations, one foetus with lumbar vertebral body no. 6 (supernumerary) and sternebra no. 3 asymmetric, one foetus with ribs no. 5 and 6 fused at base, and one foetus with sternebrae 4-6 fused and asymmetric.
[*4]: 30 mg/kg bw dose group: one foetus with hemidiaphragm. 100 mg/kg bw dose group: two foetuses with hemidiaphragm.

Ossification retardations occur scattered in the dose groups and without any trends but often with statistically significantly deviations between one dose group and controls – without any dose relationship and on litter basis all statistically significant results vanish.

* Significantly different from study controls *($p \leq 0.05$)*
** Significantly different from study controls *($p \leq 0.01$)*

Table 6.6-47. Foetal findings (main study)

Parameter		Control data		Dose (mg/kg bw/day)		
		Historical[$]	Study	10	30	100
Number foetuses			141	142	109	119
Number litters			16	15	14	14
External findings						
Foetuses affected			0	0[1)]	3[1)]	3[1)]
Litters affected			0	0[1)]	3[1)]	3[1)]
Total incidences	F	0.0 – 1.4[1)]	0	0	**2.8** *	**2.5** *
	L	*Could not be calculated*	0	0	21.4	21.4
Hind limb malrotated Pes varus position, brachydactyly, missing phalanges	F	0.0 – 0.6	0	0	0.9	
	L	0.0 – 6.3	0	0	7.1	
Hind limb hyperflexion (Arthrogryposis without skeletal finding)	F	0.0 – 0.6	0	0	0.9	
	L	0.0 – 6.3	0	0	7.1	
Multiple malformation	F	0.0 – 0.9[c]	0	0	0.9[a]	**2.5**[b]
	L	0.0 – 7.7	0	0	7.1	**21.4 0**
Visceral findings						
Foetuses affected			0	0	1	1
Litters affected			0	0	1	1

Parameter		Control data		Dose (mg/kg bw/day)		
		Historical[$]	Study	10	30	100
Diaphragm - Hernia	F	0.0 – 0.8	0	0	0.9	0.8
	L	0.0 – 6.3	0	0	7.1	7.1
Skeletal findings						
Foetuses affected			4	4	3	5
Litters affected			2	3	3	5
Total incidences	F	*No HCD range*	2.8	2.8	2.8	**4.2**
	L	*No HCD range*	12.5	20.0	21.4	**35.71**
"Runt" (small foetus <19g without malformations)						
Number small foetuses			0	2	1	1
Small foetus	F	0.0 – 4.2	0	1.4	0.9	0.8
	L	0.0 – 25.0	0	13.3	7.1	7.1

F: % foetuses,
L: % litter,
1) "runt" (small foetus <19 g) without malformations listed separately since not assessed as external malformation
*/** significantly different from study controls ($p \leq 0.05$ / $p \leq 0.01$),
$: Historical control data range from 1989 – 1995 (18 studies, 346 litters, 3225 foetuses) (data from the same laboratory, on Chinchilla rabbits, SPF quality, within ± 5 years of the current study date 1992).
a: craniochisis, dysplastic skull, protruding tongue, kyphosis, spina bifida aperta, eventration of organs, open eye, shortened extremities, bent forepaw, brachydactyly,
b: meningocele or partial acephaly, omphalocele or abdominal fissure, spina bifida occipitale (2 foetuses), malposition of limbs, brachydactyly, shortened tail (1 foetus),
c: exencephaly, open eye, arthrogryposis, brachydactyly, omphalocele, eventration of organs, spina bifida aperta

Supplementary study
There were no clear, treatment-related foetal effects in the supplementary study (Table 6.6-47.), but this could have been due to the low number of litters investigated.

Table 6.6-48. Litter responses (Caesarean section data) (supplementary study)

Parameter	Control data		Low dose 10 mg/kg	Medium dose 30 mg/kg	High dose 100 mg/kg
	Historical	Study			
Corpora lutea (total/number of dams)	2635/254= 10.4	11.5	11.8	12.6	10.7
Implantations (total/number of dams)	2545/254= 10.0	11.0	11.0	12.0	7.3**
total number of foetuses	2303	42	38	49	21
pre-implantation loss %	3.4	4.3	6.4	4.8	31.3**
post-implantation loss %	9.5	4.5	13.6	18.3*	4.5
total number of litters	254	4	4	5	3
foetuses / litter	9.1	10.5	9.5	9.8	7.0
dead foetuses / litter ratio	0	0	0	0	0
Foetal sex ratio [m/f]	1154/ 1149 =1.00				

No abnormal foetuses were discovered
* Significantly different from study controls *($p \leq 0.05$)*
** Significantly different from study controls *($p \leq 0.01$)*

Update of HCD submitted for the purpose of renewal
The main study (B.6.6.2.2.1/03) was performed from March to April 1992 and the historical control data presented in the study report cover studies conducted between January 1989 and June 1992. An update on historical control data covering also later conducted studies, i.e. from the second half of 1992 until 1995 has now been provided by the Bayer Task Force for the purpose of renewal, so that the HCD covers the period 1989 – 1995 in many of the tables. The report has been submitted as a confidential annex (annex IV) to the CLH report. The review of these updated historical control data reveals that an accumulation of these unusual abnormalities/malformations occurred within a narrow time frame of about 1-year (year 1992).

The percent litter incidences of external malformations (excluding runts) are grouped below (Figure 6.6-1.) for the years 1989 – 1991 and 1993 - 1995 respectively, and are compared with the respective incidences for the year 1992.

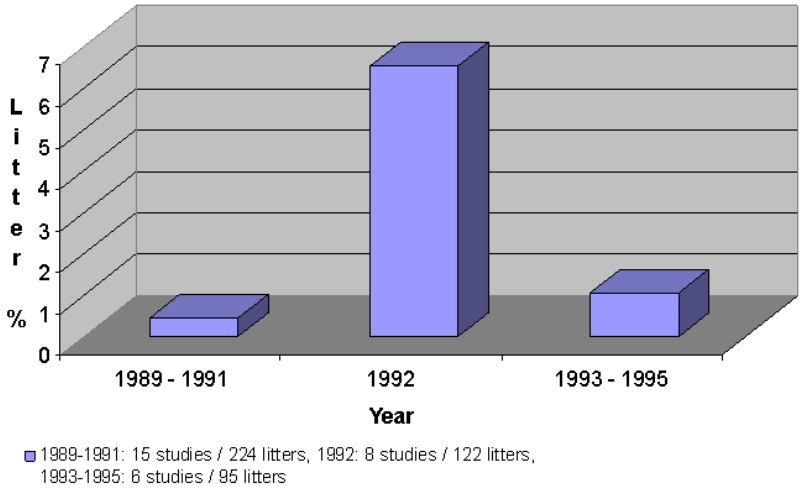

Figure 6.6-1. Mean litter incidence [%] of total external malformations (excluding runts) in control rabbits

As can be seen below, this clustering in 1992 is not only caused by the higher rate of affected studies but also by the higher incidence of total external malformations within a given study.

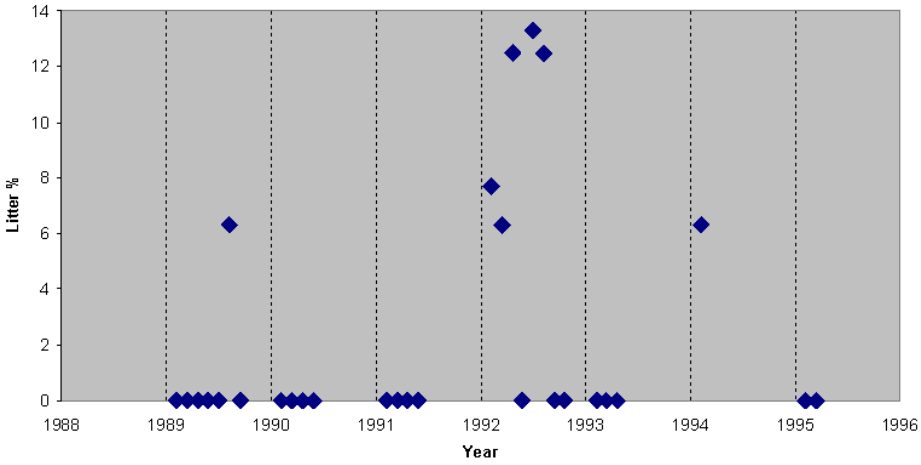

Figure 6.6-2. Mean litter incidence [%] of total spontaneous external malformations (excluding runts) per study

The incidence of external malformations in untreated controls was unusually high in the year 1992, i.e., when the tebuconazole study was performed (Figure 6.6-1.); the eight studies conducted in 1992 produced four times more cases of spontaneous external malformations (a total of 8) than all other 21 studies conducted in the three previous or following years together (a total of 2).

The most likely explanation for this transient clustering and resulting high biological variability of external malformations in 1992 is a transient impact of genetically pre-disposed rabbits in the breeder´s rabbit colony.

A re-assessment of the developmental toxicity observed in the main study from 30 mg/kg bw/day in light of the updated historical control data is therefore justified, in order to decide whether the observed three cases of external malformations (one foetus with malpositioned hind legs, one foetus with arthrogryposis, one foetus with multiple malformations; two of these were also runts) at 30 mg/kg bw/day represent a real treatment-related effect of

tebuconazole, or whether they are possibly related to the high spontaneous variability observed in the year of study conduct.

If one would assume an incidental origin of these malformations it would be important to verify that these types of malformations were also observed spontaneously in the historical controls. In fact, this is the case for all three affected foetuses at 30 mg/kg bw/day:

- Foetus No. 275 had multiple malformations, including spina bifida, eventration of liver, stomach and intestine and forepaw bent forward. The updated historical control data (page 29, (Dec 91/Jan 92)) include a very similar case of a foetus with multiple malformations, including spina bifida, omphalocele with eventration of parts of liver and intestine and bilateral arthrogryposis (= mal-positioned limb). A further historical control case of omphalocele was also seen in the updated historical control data (page 31 (Feb/Mar 92)).
- Foetus No. 72 had a malposition of the hind legs. The updated historical control data include a case with the same malformation (page 37 (May/June 94)).
- Foetus No. 232 had arthrogryposis. In the updated historical control data, there exist four historical control studies with one foetus each with arthrogryposis (page 29 (Dec 91/Jan 92), page 31 (Feb/Mar 92), page 33 (Aug/Sep 92), page 34 (Oct/Nov 92)).

Thus, all external malformations observed at the 30 mg/kg bw/day are within the range of respective historical control data, mainly from 1992, the "peak" year for spontaneous external malformations and the year in which the study was conducted. It is therefore likely that the three cases of foetuses with external malformations at 30 mg /kg bw/day represent a spontaneous event related to the high variability in spontaneous malformations observed in the year of study conduct. However, it is difficult to make a definite decision on the relationship to treatment, based on the results of this individual study without consideration of the results of the other rabbit developmental toxicity studies.

In this context, the results of the other two developmental toxicity studies that were conducted in rabbits with tebuconazole (B.6.6.2.2.1/01 and B.6.6.2.2.1/02 – summarised above) are of high significance.

The study B.6.6.2.2.1/01 gave no indications for a treatment-related effect on external malformations up to and including the highest tested dose of 30 mg/kg bw/day.

The study B.6.6.2.2.1/02 revealed the absence of any external malformations up to and including the dose of 30 mg/kg bw/day. The highest tested dose of 100 mg/kg bw/day produced a clear increase of external malformations.

UK-RMS Conclusion

There is convincing evidence that the three cases of foetuses with external malformations at 30 mg/kg bw/day in this study represent an incidental event, related to the clustering of spontaneous external malformations observed in the test facility in 1992, the year in which the study was conducted. The absence of a real treatment-related effect is confirmed by the absence of malformations at the same dose level of 30 mg/kg bw/day in two other independent rabbit developmental toxicity studies. This dose level should, therefore, be considered as a developmental NOAEL.

Overall, in this guideline oral developmental toxicity study in Chinchilla rabbits, developmental toxicity (increased post-implantation loss, decreased foetal weight and slightly increased incidence of multiple malformations) was seen at the top dose of 100 mg/kg bw/day at which maternal toxicity (reduced feed intake and body weight gain) occurred. On this basis, the NOAEL for maternal toxicity and developmental toxicity was 30 mg/kg bw/day. It should be noted that the developmental NOAEL has been raised from 10 mg/kg bw/day agreed during the first review of tebuconazole to 30 mg/kg bw/day as a review of updated historical control data provided for the purpose of renewal has shown that the malformations seen at 30 mg/kg bw/day were unrelated to treatment.

B.6.6.2.2.1c Discussion and conclusion by DK-RMS:	Regarding the conclusion drawn by UK-RMS, the DK-RMS is of the opinion that a high incidence of spontaneous total external malformation in the year 1992 could be a potential explanation, but notes that if indeed true, the malformation incidence in concurrent controls would also be expected to be high – and this was not the case. A more in-depth evaluation of the HCD would be necessary for using these data to neglect the dose–related increase of

	multiple malformations starting from 30 mg/kg bw/d and compared to the concurrent control. DK-RMS has some reservations with respect to the presented HCD data which is argumented below. *HCD* According to the Regulation No 283/2013 setting out the data requirements for active substances the historical control data shall be presented on a study by study basis giving absolute values plus percentage and relative or transformed values where these are helpful in the evaluation. If combined or summary data are submitted, these shall contain information on the range of values, the mean, median and, if applicable, standard deviation. They shall cover a five-year period, centred as closely as possible on the date of the index study. According to the applicant the main study was performed from March to April 1992. Hence, only HCD data from September 1989 to October 1994 should be included. The control data from the current study should not be included but rather used to compare with the other data. In table 6.6-47 HCD ranges of 0.0-0.9 % for foetus incidence and 0.0-7.7% for litter incidence for multiple malformation are given; this seems however to be based on 1 fetus affected in one litter in 3. study from Dec 91/Jan 92 containing 13 litters and 107 fetuses. None of the other 7 studies (or 9 if two other studies are included: 2. study nov 91/jan 92 and 1.study nov 92/jan 93) from 1992 each containing from 14-16 litters reported multiple malformation. In the concurrent control group containing 16 litters and 141 foetuses or in the low dose group (10 mg/kg bw/d; 15 litters and 142 foetuses) no external or multiple malformations were reported. The concurrent control group is of comparable size with the HCD data driving the range of 0.0-7.7%. In the concurrent study at 30 mg/kg bw/d three foetuses from three different litters were affected (one fetus with malpositioned hind legs, one fetus with arthrogryposis, and one fetus with multiple malformations) which raises concerns. At 100 mg/kg bw/day three foetuses from three different litters had multiple malformations. Figure 6.6-1 illustrates a range of 0.0 to circa 6% mean litter incidence for total external malformations (the concurrent control group of the tebuconazole study is included in the mean value; however, two studies: 2. study from nov 91 /jan 92 and 1. study from nov 92/jan 93 are not included; all three studies had no malformed foetuses with external malformations). Regarding the Tabel 6.6-47 the HCD ranges of 0.0-4.5% of total fetus incidence of external findings ("malformed foetuses" in HCD data) is referring to a historical control study just outside the acceptable 5 years centred on the date of the index study. In addition, the value of 4.5 includes runts without malformations and should therefore not be compared to values where runts without malformation have been exluded. The total fetus incidence of external findings should instead be corrected to 0-1.4% which is the maximum fetus incidence with external findings without runts (4.study 92 aug/sep). It is also noted that it seems the HCD ranges in general have included the control group of the concurrent study which is inappropriate. According to ECETOC (Monograph No. 31 2002) and Moore et al. 2013 runts are considered of high concern on their own and listed under external abnormalities and malformations. It could be discussed whether runts should be taken out of the external findings as proposed by applicant and accepted by UK-RMS in Table 6.6-47. In consideration of the large historical control database, the individual specific external malformations seem to be rare spontaneous events and typically observed in 1 foetus in one litter. It could be argued that it would seem unlikely that 3 fetuses from 3 litters with external malformations arising as spontanoues events should then be detected in the current study at 30 mg/kg bw/d and also considering that treatment related malformations

	are seen in the highest dose. Statistical significance does not need to be present to validate the biological significance of treatment-related effects. This is particularly true of findings with low incidence (i.e., rare malformations) or high variability, or in situations where the concurrent control data have an unusual incidence profile (OECD GD 43, 2008). It is mentioned by UK-RMS that the study B.6.6.2.2.1/01 gave no indications for a treatment-related effect on external malformations up to and including the highest tested dose of 30 mg/kg bw/day. However this study was of low reliability and was only accepted as supplementary information, due to the poor reporting, reduced database being available for inspection, doses tested being low (not tested up to maternal toxicity) and increased number of losses not being commented. *Conclusion:* The DK-RMS hence finds it plausible that the severe malformations seen at 30 mg/kg are of biological significance and treatment related also considering that malformations were found in the following high dose. Therefore NOAEL should be kept as for the initial review at 10 mg/kg bw/d.

d)

Previous evaluation	In DAR (2006) for original approval

Study ID	B.6.6.2.2.1/04
Study title	HWG 1608 (c.n. Tebuconazole). Mechanistic study on embryotoxic effects in rabbits after oral administration
Matrix ID	26
Study dates	March to July 1999
Test substance	(HWG 1608) Tebuconazole
Purity (%) **Batch no.**	98.5 278679012
Test animals	Mated female CHB-W (chinchilla) rabbits (mated until 2 times copulation had been observed)
Groups	14 controls – 15 dosed animals
Dose	0 or 100 mg/kg bw
Route	Oral by gavage Treated daily from day 6 – 19 p.c.
Vehicle	0.5 % Cremophor EL in demineralised water. Total volume applied 4 mL/kg
GLP	Yes, but determination of test compound in maternal plasma and foetal tissue as well as biochemical /toxicological investigations in maternal liver and adrenals were not performed in compliance with GLP principles
Guideline	Not a guideline study, but dosing of the animals and most examinations were performed by methods comparable to the OECD guideline 414 "Teratogenicity" (1981). The OECD guideline 414 has been revised in 2001 with extension of the dosing period to cover more than the organogenesis period and a few minor changes.
Deviation	Not applicable.
Acceptable	Acceptable, as an investigative study.
NOAEL	N/A – investigative study.
Effects at the tested dose	**Effects at the tested dose:** Maternal: reduced food consumption, decrease in overall corrected bw gain in dams however not statistically significant in dams (weight loss day 6-10 p.c. only). Developmental: statistically significantly decreased foetal weight

Methods
No test guideline was referred to but administration of tebuconazole took place in a manner similar to OECD

guideline 414 “Teratogenicity”. This was a mechanistic study conducted to investigate further the maternal and developmental toxicity induced by tebuconazole.

Chinchilla rabbits (CHB-W) were used for this study. Fourteen mated females were used as controls and 15 mated females were given daily doses of 100 mg/kg bw tebuconazole (98.5 % pure) suspended in 0.5 % Cremophor EL in demineralised water by gavage on days 6 to 19 of gestation. Control animals were given 0.5 % Cremophor EL in demineralised water. The animals were inspected at least once daily for mortality, clinical signs and behaviour. The feed intake was recorded as were body weights at regular intervals. On day 19 of gestation the dams were sacrificed 2 hours after the last dosing. Blood was drawn from an extremity vein just prior to this. Caesarean section was performed on all females and reproductive parameters were recorded. The liver, the ovaries (pairwise), the adrenals (pairwise) and the placentas of all pregnant females (surviving) were weighed. The right liver lobes and two placentas with foetuses from each of the pregnant females were fixed in buffered 4 % formaldehyde. The same happened to the adrenals and ovaries of 3 females from each group. Enzyme activities of the livers were determined. The blood samples were analysed for content of tebuconazole, as were foetal tissues.

Results

Maternal effects

Two animals died during the study due to intubation errors. Dosing did not affect appearance or behaviour of the animals. Feed consumption was decreased (statistically significant and 35 % change compared to control, from days 6 - 12) and weight loss was recorded (statistically significant from day 6 - 10) (Table 6.6-48.). Correspondingly amounts of faeces, water consumption and urination were decreased in treated animals.

Table 6.6-49. Maternal effects

Parameter	Control data		100 mg/kg
	Historical	Study	
Number of dams examined		13	12
Clinical findings during application of test substance		0	0
Abortions		0	0
Body weight gain			
day 6-10		1.4	-86.0**
day 0-end of test		73.5	7.4
Food consumption			
day 6-12		275.3	**179.6****
(%)[a]		-	***(-35)***
Pregnancies %		92.9	92.3

* Significantly different from study controls *($p \leq 0.05$)*

** Significantly different from study controls *($p \leq 0.01$)*

(%)[a] percent change compared to control

Necropsy in females did not reveal treatment-related findings (at 100 mg/kg bw/day). The absolute and relative weights of liver and adrenals, and the absolute placental weight in the treated animals did not differ to a statistically significant extent from the control group values (Table 6.6-49.).

The absolute and relative weights of ovaries were decreased when compared to the control value (> 10 % change compared to control) (Table 6.6-49.). However, histopathology did not reveal treatment-related findings. Therefore, and due to a lack of statistical significance, the decreased ovary weight was not considered treatment-related. A marginal decrease was also seen in the placental weight but histopathology did not reveal treatment related findings.

The adrenals showed a distinct hypertrophy of the cortical cells of the zona fasciculata (2/3 at 100 mg/kg bw/day compared to 0/3 in controls) (Table 6.6-49.) accompanied by a slightly increased activity of 11ß-hydroxylase (22 % change compared to control) in the adrenal mitochondria and a slightly increased concentration of 11-deoxycorticosterone (20 % change compared to control) and corticosterone (22 % change compared to control) in the adrenal tissue (all not statistically significant) (Table 6.6-50.). The concentration of cortisol in the adrenal tissue was also marginally increased (10 % change compared to control); however, this increase in cortisol was due to one female only with a high concentration and therefore it is not considered treatment-related.

Liver enzyme induction (≥ 10 % change compared to control, mainly 7-ethoxycoumarin deethylase; ß-

hydroxylation and androstenedione formation in the testosterone metabolism assay) was observed in the treated animals (Table 6.6-51.). This correlated microscopically with centrilobular cytoplasmic change in single females. A statistically significant decrease in glutathione-S-transferase activity was evident (28 % change compared to control), which could be indicative of impaired liver function.

Table 6.6-50. Organ weights and incidence of histopathological findings in females

	Females	
Dose [mg/kg bw/day]	**0**	**100**
Liver		
Absolute weight [g]	106.11	103.72
Relative weight [%]	2.5831	2.5813
Histopathological findings:		
cytoplasmic change	0/14	2/13
vacuolation/hepat.	5/14	2/13
periport. inflammation	8/14	8/13
glycogen increased	4/14	4/13
congestion	1/14	0/13
fatty change hepat.	11/14	7/13
fatty change fat st.	3/14	5/13
Ovaries		
Absolute weight [g] *(%)[a]*	0.983 -	0.829 *(-16)*
Relative weight [%] *(%)[a]*	0.0240 -	0.0206 *(-14)*
Histopathological findings:		
No. c.l. of pregnancy	0/3	1/3
Adrenals		
Absolute weight [g]	0.289	0.301
Relative weight [%]	0.0070	0.0075
Histopathological findings:		
Hypertrophy zona fasciculata	0/3	**2/3**
Placenta		
Absolute weight [g] *(%)[a]*	3.80 -	3.66 *(-3.7)*
Histopathological findings:		
foc. mineralization	13/13	12/12

(%)[a] percent change compared to control

Table 6.6-51. Concentrations of some steroids in maternal adrenal tissues and mitochondria

Compound	**0 mg/kg bw/day**	**100 mg/kg bw/day**
Cortisol (nmol/adrenal)	4.0	4.4
(%)[a]	-	*(10)*
Corticosterone (nmol/adrenal)	6.5	7.9
(%)[a]	-	*(22)*
11-Deoxycorticosterone (nmol/adrenal)	2.0	2.4
(%)[a]	-	*(20)*
Progesterone (nmol/adrenal)	1.1	1.1
(%)[a]	-	*(±0)*
11-β-hydroxylase (nmol/adrenal)	2.15	2.63
(%)[a]	-	*(22)*

(%)[a] percent change compared to control

Table 6.6-52. Group mean values of the enzyme activities in the maternal liver

Enzyme	0 mg/kg bw/day	100 mg/kg bw/day
ECOD (nmol/g/min)	22.5	34.9*
(%)[a]	-	*(55)*
EROD (nmol/g/min)	1.56	2.08
(%)[a]	-	*(33)*
ALD (nmol/g/min)	212.6	253.0
(%)[a]	-	*(19)*
EH (nmol/g/min)	4182	4952
(%)[a]	-	*(18)*
GS-T (nmol/g/min)	825	597*
(%)[a]	-	*(28)*
GLU-T (nmol/g/min)	2319	2556
(%)[a]	-	*(10)*

* statistically significant difference to control $p < 0.05$
(%)[a] percent change compared to control

Table 6.6-53. Results of the testosterone metabolism assay in the maternal liver

Enzyme	0 mg/kg bw/day	100 mg/kg bw/day
6α-hydroxylation	0.9	0.8
(%)[a]	-	*(-11)*
7α-hydroxylation	n.d.	n.d.
(%)[a]	-	*(±0)*
6β-hydroxylation	49.3	62.2
(%)[a]	-	*(26)*
16α-hydroxylation	19.8	16.1
(%)[a]	-	*(-19)*
16β-hydroxylation	4.9	6.5
(%)[a]	-	*(33)*
2α-hydroxylation	0.4	0.1
(%)[a]	-	*(-75)*
2β-hydroxylation	8.1	8.1
(%)[a]	-	*(±0)*
androstenedione formation	79.7	107.1
(%)[a]	-	*(34)*

n.d. not detectable, below limit of quantification
(%)[a] percent change compared to control

Developmental toxicity
Foetal weights were statistically significantly depressed (12 % change compared to control) but the external appearance of the foetuses was not affected by treatment. Number of live foetuses was reduced, but not to a statistically significantly level (also < 10 % compared to control) (Table 6.6-53.). No examination of the foetuses was performed.

Table 6.6-54. Litter response (Caesarean section data)

Parameter	Control data	100 mg/kg
Corpora lutea (total/number of dams)	156/13 = 12.0	140/12 = 11.7
Implantations (total/number of dams)	141/13 = 10.8	122/12 = 10.2
Resorptions (total/number of dams)	0	0
Total number of foetuses	132	113
Pre-implantation loss %	9.6	12.9
Post-implantation loss %	6.4	7.4
Total number of litters	14	13
Live foetuses / litter (ratio)	132/13 = 10.2	113/12 = 9.4
(%)[a]	-	*(-8)*
Dead foetuses / litter (ratio)	0	0

Parameter	Control data	100 mg/kg
Foetus weight (mean) [g]	2.27	2.00**
(%)[a]	-	*(-12)*
Placenta weight (mean) [g]	3.80	3.66

* Significantly different from study controls *($p \leq 0.05$)*
** Significantly different from study controls *($p \leq 0.01$)*
(%)[a] percent change compared to control

Toxicokinetic investigations
In a preliminary toxicokinetic screening experiment after a single oral administration of 100 mg/kg bw/day to pregnant rabbits, the plasma concentrations were measured after 1, 2, 4, 7, and 24 hours. The tebuconazole peak concentration was reached after 1 hour. After 7 hours the plasma concentrations were lowered only by 25 – 30 %. After 24 hours tebuconazole was not detectable.

Based on these preliminary study results, in the present study blood samples were taken 2 hours after the last administration on gestation day 19 in 14 control and 13 treated dams and foetal tissue (one foetus per litter was homogenized) from 3 control and 12 treated dams was sampled. The mean tebuconazole concentration in plasma of treated dams was 2.66 µg/mL (1.01 - 5.72 µg/mL) and in foetal tissue 2.3 µg/g (0.83 - 4.06 µg/g).

The mean unbound plasma concentration of tebuconazole was calculated taking into account the results of the plasma protein binding study (B.6.1.2/03). In this study the unbound fractions of tebuconazole ranged between 3.24 % in mouse and 5.82 % in rat. The fraction unbound in human plasma amounted to 5.09 %. There was no significant concentration dependency of the plasma protein binding for all species in the tested concentration range.

The mean total plasma concentration of 2.66 mg/L measured in pregnant rabbits corresponds to the mean unbound plasma concentration of tebuconazole of 0.15 mg/L (0.06 - 0.32 mg/L).

DK RMS Table with body weight calculations

ID	Reference	Species	Body weight data discussion
26	B.6.6.2.2.1/04	Rabbit	Doses: 0, 100 mg/kg Dose / Body weight gain (g) (days p.c) mg/kg b.w./day: 0 — mean 6-10: 1.4 — 6-19: -12.7 — 0-19: 73.5 — corrected[a] 0-19: -106.6 mg/kg b.w./day: 100 — mean 6-10: -86.0** — 6-19: -35.7 — 0-19: 7.4 — corrected[a] 0-19: -159.4 **statistically significant difference to control p<0.01 a Corrected body weight gain was determined by subtracting the uterus weight on day 19 p.c. from the body weight gain from day 0 to 19 p.c. As evident from the table above a statistically significant weight loss occurred in the females after start of treatment (day 6 to 10 p.c.), which resulted in marginally decreased overall weight gain during the treatment period and in a decreased corrected body weight gain at the 100 mg/kg level. When looking in more detail on the provided data in the study report it becomes apparent that before dosing starts (day 0-6) the control dam gain 86 gram, while the exposed group only gain 43 grams, which could explain some of the observed differences between the two groups. During the first 4 days of exposure, the exposed dams loose 86 grams, but during the next 9 days these dams gain 60 gram, while the control dams loose 11 grams in the same period. Hence, there are some signs of maternal toxicity in the exposed animals during the beginning of the exposure, but the severity is difficult to assess, and it doesn't seem to be very marked since the decrease in overall bw gain and corrected bw gain was not statistically significant.

			Food consumption was significantly reduced in the exposed group from GD 6-12. At necropsy the absolute and relative weight of liver, adrenals and ovaries was not statistically significant changed compared to controls liver weight. Ovaries and adrenal were not significantly affected. The absolute and relative weights of the ovaries (>10% compared to control) were decreased when compared to the control value. Histopathology of the ovaries did, however, not reveal treatment related findings.

UK-RMS Conclusion
In this non-standard developmental toxicity study in Chinchilla rabbits, focusing on investigations of maternal toxicity, a dose of 100 mg/kg bw/day tebuconazole produced maternal toxicity consisting of reduced food consumption, decreased body weight gain, liver hypertrophy with associated enzyme induction and hypertrophy of the adrenal cortical cells which was associated with slight increases of 11-deoxycorticosterone and corticosterone in the adrenal gland. Foetal weight was also decreased, but no further foetal examinations were performed. The applicant (Bayer Task Force) postulates that the malformations seen in rabbits at 100 mg/kg bw/day in previous studies could be the consequence of the adrenal toxicity and the elevated levels of some glucocorticoids seen in this study. However, as no foetal examinations were performed in this study and considering that the increased levels of some glucocorticoids were marginal, this remains only a hypothesis.

B.6.6.2.2.1d Discussion and conclusion by DK-RMS:	It is well established that steroid hormone synthesis is affected by tebuconazole (see section B.6.8.3.1.3). While such changes to the steroidogenesis enzymes could occur in the adrenal gland, the adrenal glands in this study only showed a non-significant (4%) increase in weight. It therefore seems unlikely that this should be the driving effect for the tebuconazole induced changes in the offspring. Changes in steroid hormone synthesis in maternal ovaries (which were 14-16 % decreased) is a more plausible explanation.

B.6.6.2.2.2. Summary of rabbit developmental studies

The potential for tebuconazole to adversely affect development in the rabbit was investigated in four studies, using two different strains of rabbit (Himalayan and Chinchilla rabbits). Three of the four studies were conducted according to GLP and OECD test guidelines; the fourth study was a non-standard study investigating maternal toxicity in more detail.

Developmental toxicity (increased resorptions) started to occur from 30 mg/kg bw/d. At 100 mg/kg bw/d there was also decreased foetal weight and a slightly increased incidence of external malformations, including cleft palate (1 foetus), malrotation of hind limb, hemimelia and agenesis of claws. An overall NOAEL of 10 mg/kg bw/d was identified for developmental toxicity. Maternal toxicity (decreased body weight gain) also started to occur from 30 mg/kg bw/d, becoming more severe (body weight loss, decreased food consumption, liver and adrenal hypertrophy and increased levels of corticosteroid hormones) at 100 mg/kg bw/d. An overall NOAEL of 10 mg/kg bw/d was also identified for maternal toxicity. It is possible that some of the developmental effects caused by tebuconazole in the rabbit were secondary to the observed maternal toxicity.

B.6.6.2.2.2. Overall summary of rabbit developmental	Developmental toxicity (increased resorptions) started to occur from 30 mg/kg bw/d (B.6.6.2.2.1/01, B.6.6.2.2.1/02, B.6.6.2.2.1/03). At 100 mg/kg bw/d there was also decreased foetal weight and a slightly increased incidence of external malformations, including cleft palate, malrotation of hind limb, hemimelia and agenesis of claws. B.6.6.2.2.1/02 observed a marginally decreased foetal body weight (6 % change compared to control), which correlated with slightly retarded ossification. In addition, an

studies by DK-RMS:	increased incidence of external malformations occurred at 100 mg/kg bw/day, including cleft palate, malrotation of hind limb, enlarged fontanelle, hemimelia and agenesis of claws (total incidence of 33.3 at 100 mg/kg bw/day compared to 0 in control). Cleft palate was seen in 1.1% of the fetuses in 6.7% of the litters at the high dose. At a dose-response pattern was seen in the incidence of skeletal findings being statistically significant at 100 mg/kg bw/day, but starting at the low dose of 10 mg/kg bw/day. In B.6.6.2.2.1/03 an increased incidence of malformations is seen at 30, but UK_RMS argues that this is due to higher background levels in the period of performing this study. DK_RMS finds that it is plausible that effects at 30 are exposure related, as no malformations were seen in controls and low dose of 10 mg/kg bw/d. An overall NOAEL of 10 mg/kg bw/d was identified for developmental toxicity. Maternal toxicity (decreased body weight gain) also started to occur from 30 mg/kg bw/d, becoming more severe (body weight loss, decreased food consumption, liver and adrenal hypertrophy and increased levels of corticosteroid hormones) at 100 mg/kg bw/d. An overall NOAEL of 10 mg/kg bw/d was also identified for maternal toxicity. The developmental effects cannot by default be consided related to unspecific maternal toxicity. Further discussion on possible relations with maternal effects is presented below in relation to CLP criteia. DK RMS notes that in general, reductions in maternal body weight gain may be related to reduced food intake during early days of pregnancy and/or to specific toxicity to uterine and fetal growth. Therefore, on a case-by-case basis a thorough evaluation of relationships between maternal weight gain changes and fetal growth and survivial needs to be carried out. DK RMS considers that it cannot be demonstrated that effects are secondary to marked systemic toxicity. In contrast, effects on mothers are likely related to specific modes of action causing developmental toxicity.

B.6.6.2.3. *Mice*

Three developmental toxicity studies by oral administration in the mouse were described in the original DAR (2006) (B.6.6.2.3.1/01; B.6.6.2.3.1/02 and B.6.6.2.3.1/03). The study B.6.6.2.3.1/02 served as a supplementary study and whilst NOAELs were not derived an effect on maternal toxicity was clear at the top dose.

One developmental toxicity study by dermal administration in the mouse was described in the original DAR (2006) (B.6.6.2.3.2/01).

No new developmental toxicity studies in the mouse were submitted for the purposes of renewal.

B.6.6.2.3.1. Developmental toxicity study following oral administration in mice

a)

Previous evaluation	In DAR (2006) for original approval

Study ID	B.6.6.2.3.1/01
Study title	HWG 1608 – Study for embryotoxic effects on mice following oral administration
Matrix ID	27
Test substance	(HWG 1608) Tebuconazole
Purity (%) **Batch no.**	93.6 - impurities: 5.5 symmetric isomer the (purity is not according to the specifications especially caused by a high content of a symmetric isomer) 1616002/84
Test animals	Mated female NMRI/ORIG Kisslegg mice (mated until vaginal plug appeared (day zero of gestation))
Groups	25/dose

Dose	0, 10, 30 or 100 mg/kg bw/day on day 6-15 post mating
Route	Oral by gavage
Vehicle	0.5% aqueous Cremophor EL solution. Total volume applied was 5 mL/kg bw
GLP	Yes
Guideline	Pesticide Assessment Guidelines Subdivison F, Hazard Evaluation: Human and Domestic Animals, EPA, 83-3, "Teratogenicity Study", Revised Edition (1984) which is in accordance with OECD guideline 414 "Teratogenicity" (1981). Both the EPA and the OECD guidelines have been revised (OECD guideline 414 in 2001) with extension of the dosing period to cover more than the organogenesis period and a few minor changes
Deviation	In relation to the version of OECD guideline 414 (1981) the only deviation is that reporting is very brief, which is found not to compromise the results The following deviations from the OECD-Guideline 414 (2001) occurred: - Dosing was performed during the period of organogenesis. - Reporting deficiencies: Food consumption, uterus weight, number of corpora lutea, number / percent pre-implantation loss not reported. - Less than 50 % of foetuses examined for visceral alterations. - The validity of the study is partially compromised.
Acceptable	Acceptable
Historical control information	Date: 1991 Report No. 97411 Period: 1983 – 1989 Species: Mouse Strain: NMRI HCD is combined from B.6.6.2.3.1/03 and two other laboratories
NOAEL	Maternal toxicity: No maternal toxicity recorded up to 100 mg/kg bw/day (the top dose). Developmental toxicity: 10 mg/kg bw/day.
Effects at the LOAEL	Developmental toxicity: Increased number of runts from 30 mg/kg bw/day; increased number of malformations and increased placental weights in the high dose group (100 mg/kg bw/day)

Methods

In a developmental toxicity study in accordance with OECD 414 (1981) and EPA Pesticide Assessment Guidelines, Subdivision F, 83-3, "Teratogenicity Study", November 1984 and under the principles of Good Laboratory Practice, groups of 25 inseminated NMRI mice were given by gavage daily doses of 0, 10, 30 or 100 mg tebuconazole (93.6 % pure)/kg bw on days 6 - 15 after mating. The test substance was suspended in 0.5 % Cremophor EL aqueous solution. The dams were inspected daily with respect to mortality, appearance and behaviour and were weighed daily. On day 18 of gestation the foetuses were obtained by caesarean section and examinations were performed on the foetuses and the uteri to determine possible embryotoxic and teratogenic effects of the dosing. Numbers of implantations, live and dead foetuses and resorptions were recorded and the foetuses were weighed individually and sexed. The placentas were weighed and the weight recorded. The foetuses were examined morphologically for possible internal and external abnormalities. Around 30 % of the foetuses were stained using the modified Wilson technique and examined for visceral malformations and the rest of the foetuses were stained with Alizarin Red S after clearing with potassium hydroxide solution (Dawson technique) for evaluation of the bone system.

Results

Maternal toxicity:

No overt signs of maternal toxicity at any dose were observed. Treatment with tebuconazole did not hinder weight development in the pregnant animals (< 10 % change compared to control) (Table 6.6-55.).

Table 6.6-55. Maternal effects

Parameter	Dose (mg/kg bw/day)					
	0 (control)	10		30		100
			(%)[a]		*(%)*[a]	*(%)*[a]
Main study						

Parameter	Dose (mg/kg bw/day)						
	0 (control)	10		30		100	
			(%)[a]		*(%)[a]*		*(%)[a]*
Number of dams examined	24	23		23		20	
Clinical findings during application of test substance	0	0		0		0	
Mortality of dams (%)	0	0		0		0	
Body weight gain (g)							
Day 6-15	12.8	13.4	*(+5)*	13.7	*(+7)*	13.4	*(+5)*
Day 0-end of test (18)	23.0	24.9	*(+8)*	24.6	*(+7)*	24.9	*(+8)*
Pregnancies (%)	100	95.7		100		100	
Necropsy findings in dams dead before end of test	0	0		0		0	

(%)[a] percent change compared to control

Developmental toxicity

The dose of 10 mg/kg bw/day was tolerated without any effects on the intrauterine development. The mid-dose of 30 mg/kg bw/day resulted in a statistically significant increased incidence of small foetuses (20 small foetuses/runts compared to 5 in the control, 300 % change compared to control) (Table 6.6-58.). Although mean foetal body weight at this dose (1.37 g) was comparable to that in the control group (1.36 g) (Table 6.6-56.) foetal development was clearly slightly delayed at 30 mg/kg bw/day.

The top dose of 100 mg/kg bw/day resulted in a statistically significantly increased incidence of runts (26 small foetuses/runts compared to 5 in controls, 420 % change compared to control) (Table 6.6-58.). In addition, mean foetal weight (1.30 g) was decreased compared to controls (1.36 g) (Table 6.6-56.), delayed ossification and skeletal retardations/anomalies were marginally increased and placental weights were also slightly increased (10 % change compared to control) (Table 6.6-56.). A statistically significantly increased number of external malformations occurred at 100 mg/kg bw/day (Table 6.6-57). The number of foetuses with cleft palate was increased (> 10 % change compared to control) (Table 6.6-58.). Cleft palate is a common malformation in this strain of mice, however incidence at 100 mg/kg bw/day were outside of the range of the HCD provided. Other malformations and anomalies (face malformations, kinked and shortened tail, dilation of brain ventricles, vertebral asymmetry, spinal dysplasia, rib fusion, partial aplasia of parietal bone) were also increased above controls and historical control data (> 10 % change compared to control at 100 mg/kg bw/day) (Table 6.6-58.).

Table 6.6-56. Intrauterine development

Parameter	Control data		Dose (mg/kg bw/day)		
	Historical[b]	Study	10	30	100
Implantations (total/number of dams)		255/24 = 10.6	248/23 = 11.2	246/23 = 10.7	229/20 = 11.4
Resorptions (total/number of dams)	0.7 – 1.7	19/24 = 0.8	0.7[b)]	12/23 = 0.5	1.4
Early	0.0 – 1.0	0.1	0.0	0.0	0.0
Late	0.4 – 1.6	0.7	0.7	0.5	1.4
Total number of foetuses		236	234	234	202
Post-implantation loss [%]		7.5	9.3	4.9	11.8
Total number of litters		24	23	23	20
Foetuses / litter		9.8	10.2	10.2	10.1
Dead foetuses / litter ratio		0	0	0	0
Foetus weight (mean) [g]	1.12 – 1.36	1.36	1.37	1.37	1.30
Placenta weight (mean) [g] *(%)[a]*	0.09 – 0.10	0.10	0.10	0.10	**0.11*** ***(10)***
Foetal Sex Ratio [M/F]		121/115	127/107	112/122	96/106

* Significantly different from study controls *($p \leq 0.05$)*

** Significantly different from study controls *($p \leq 0.01$)*

a) Historical control data range from 1983 – 1989 (6 studies) from performing laboratory and in same strain.

b) one female had a total litter loss (7 early resorptions) and was excluded from the mean calculations

(%)[a] percent change compared to control

Table 6.6-57. Examination of the foetuses

Parameter	Control data		Dose (mg/kg bw/day)		
	Historical	Study	10	30	100
External malformations[*1] [%]	0.7	0.4	1.8	0.0	**6.4***
(%)[a]	-	-	*(350)*	*(-100)*	***(1500)***
Skeletal anomalies[*2] [%]	0.5	0.8	0.4	0.9	**4.0**
(%)[a]	-	-	*(-50)*	*(13)*	***(400)***

[*1]: Malformations in historic controls are: six findings of cleft palate, and one finding each of rib fusion, "open eye" (+ other anomalies), exencephaly (+ other anomalies), and tail anomaly. In this study: Control group: one finding of cleft face/jaw/pale (+ other anomalies), 10 mg/kg bw group: four findings of cleft palate (2 in combination with other anomalies), and 100 mg/kg bw group: Six findings of cleft palate (one combined with micrognathia and one finding of each of the following: dilation of brain ventricle, vertebral asymmetry, kinked and shortened tail, partial aplasia of the parietal bone, rib fusion (and floating rib, spinal kink), and 2 finding of spinal dysplasia.
[*2]: Control group: one foetus with slight cleft in sternum + rudimentary skull ossification centres, and one with missing hyoid bone ossification centres.
10 mg/kg bw group: one foetus with missing hyoid bone ossification centres.
30 mg/kg bw group: two foetuses with rudimentary skull ossification centres.
100 mg/kg bw group: Eight foetuses with changes: Five foetuses with rudimentary skull ossification centres, two foetuses with missing, separated ossification centres of the hyoid bone, and one foetus with vertebrae spine.
* Significantly different from study controls ($p \leq 0.05$)
** Significantly different from study controls ($p \leq 0.01$)
(%)[a] percent change compared to control

Table 6.6-58. Foetal findings

Parameter		Control data		Dose (mg/kg bw/day)		
		Historical [a)]	Study	10	30	100
Number foetuses		1421	236	234	234	202
Number litters		133	24	22 [b)]	23	20
External malformations						
Foetuses affected		-	1 [c)]	4	0	**13**
Litters affected		-	1	2	0	**8**
Total incidences	F	0.0 – 1.4	0.4 [c)]	1.7	0	**6.4** *
	L	0.0 – 11.0	4.2	9.1	0	**40.0**
Cleft palate	F	0.0 – 2.0	0.4 [c)]	1.7	-	**3.0**
	L	0.0 – 20.0 0.0 – 32.0[##]	4.2	8.7	-	**20.0**
Face malformations [d)]	F	0.0 – 0.4	0	0	0	0.5
	L	0.0 – 4.2	0	0	0	5.0
Tail anomaly	F	0.0 – 0.4	0.4 [c)]	-	-	0.5
	L	0.0 – 4.8	4.2	-	-	5.0
Skeletal anomalies						
Cerebral ventricle enlarged	F		0	0	0	0.5
	L		0	0	0	5.0
Vertebral asymmetry	F		0	0	0	0.5
	L		0	0	0	5.0
Spinal dysplasia	F	*No HCD*	0.4 [c)]	-	-	1.0
	L		4.2	-	-	5.0
Os parietale partial aplasia	F	0.0 – 1.3	-	-	-	0.5
	L	0.0 – 8.3	-	-	-	5.0
Ribs fused or deformed	F	*No HCD*	0.4 [c)]	0.4	-	0.5
	L		4.2	5.0	-	5.0
"Runt" (small foetus <-2 standard deviation of the control)						
Number small foetuses		-	5	4	**20***	**26***
Small foetuses	F	*no HCD*	2.1	1.7	8.6	11.9
	L	*no HCD*	16.7	13.6	43.5	50.0

Parameter	Control data		Dose (mg/kg bw/day)		
	Historical [a)]	Study	10	30	100

F: % foetuses, L: % litter,
- no information available
*/** significantly different from study controls ($p \leq 0.05$ / $p \leq 0.01$)
[a)]Historical control data range from 1983 – 1993 (11 studies, 247 litters, 2760 fetuses) and public literature, HCD supplied as supplement to study, covering period from 1983 – 1989, species mouse, strain NMRI, 6 studies, number of females examined 133.
[b)]one female had a total litter loss (7 early resorptions) and was excluded from the mean calculations (non pregnant with at least one viable foetus)
[c)]one foetus with multiple malformations
[d)]Cheilognathopalatochisis
According to public literature from 1969 (Peters and Strassburg, 1969). Not considered appropriate by DK-RMS due to the data being obtained very much outside a 5 year period centered around the B.6.6.2.3.1/01 study as well as the HCD seem to be from mice exposed to noice as a stress factor during gestation.

DK RMS Table with body weight calculations

ID	Reference	Species	BW discussion
27	Study B.6.6.2.3.1/01	Mouse	- Doses: 0, 10, 30, 100 mg/kg bw/day - GD 6-15 - In this study maternal bw gain during exposure and from day 0 to the end of test was not affected.

UK-RMS Conclusion
In this GLP and guideline "teratogenicity" study in NMRI mice, there were no signs of maternal generalised toxicity up to and including the top dose of 100 mg/kg bw/day. The NOAEL for maternal toxicity was therefore 100 mg/kg bw/day. It is noted that this is inconsistent with the maternal effects seen in subsequent developmental toxicity studies in the mouse. There was an increased number of runts from 30 mg/kg bw/day and reduced foetal weight, delayed ossification and an increased number of external malformations (including cleft palate and tail abnormalities) and anomalies at the top dose (100 mg/kg bw/day). No adverse effects on development occurred at the low-dose level of 10 mg/kg bw/day. On this basis, the NOAEL for developmental toxicity is 10 mg/kg bw/day.

B.6.6.2.3.1a Discussion and conclusion by RMS-DK:	The DK-RMS agrees with the UK-RMS conclusion. HCD HCD was included in the study report from 6 studies from 1983-1989 from performing laboratory and in same species and strain (NMRI mice). HCD from 2 more studies from the performing laboratory and in the same strain was available in the study report from B.6.6.2.3.1/03. In addition there were HCD from 3 studies from another laboratory also in NMRI mice. The HCD from the performing laboratory and conducted within the 5 year interval around B.6.6.2.3.1/01 is considered the most relevant, and is shown in the table below.

Parameter		Dose (mg/kg bw/day)				HCD Performing laboratory, same strain, 1983-1989 (6 studies, 133 litters, 1421 fetuses)	Historical control data range from 1983 – 1993 (11 studies, 247 litters, 2760 fetuses), data combined from 2 different laboratories (NMRI mice)
		0	10	30	100	% range	% range
Number foetuses Number litters		236 24	234 22 [b)]	234 23	202 20		
External examination							
Foetuses affected		1 [c)]	4	0	**13**		
Litters affected		1	2	0	**8**		
Total incidences	F	0.4 [c)]	1.7	0	**6.4** *	0.0 – 1.4	0.0 – 2.5
	L	4.2	9.1	0	**40.0**	0.0 – 11.1	0.0 – 26.1
Cleft palate	F	0.4 [c)]	1.7	-	**3.0**	0.0-0.97	0.0 – 2.0
	L	4.2	8.7	-	**20.0**	0.0- 5.56	0.0 – 20.0
Face malformations [d)]	F	0	0	0	0.5	0.0 – 0.4	0.0 – 0.4
	L	0	0	0	5.0	0.0 – 4.2	0.0 – 4.2
Tail anomaly	F	0.4 [c)]	-	-	0.5	0.0 – 0.4	0.0 – 0.4
	L	4.2	-	-	5.0	0.0 – 4.8	0.0 – 4.8
Visceral examination							
Brain ventricles dilated	F	-	-	-	0.5	0.0 – 0.4	0.0 – 0.4
	L				5.0	0.0 – 5.0	0.0 – 5.0
Skeletal examination							
Cerebral ventricle enlarged	F	0	0	0	0.5		
	L	0	0	0	5.0		
Vertebral asymmetry	F	0	0	0	0.5		
	L	0	0	0	5.0		
Spinal dysplasia	F	0.4 [c)]	-	-	1.0		
	L	4.2	-	-	5.0		
Os parietale partial aplasia	F	-	-	-	0.5		0.0 – 1.3
	L	-	-	-	5.0		0.0 – 8.3
Ribs fused or deformed	F	0.4 [c)]	0.4	-	0.5	0.0 – 0.4 [c)]	
	L	4.2	5.0	-	5.0	0.0 – 4.2	
"Runt" (small foetus <-2 standard deviation of the control)							
Number small foetuses		5	4	**20***	**26***		
Small foetuses	F	2.1	1.7	8.6	11.9		
	L	16.7	13.6	43.5	50.0		

F: % foetuses, L: % litter,
- no information available
*/** significantly different from study controls ($p \leq 0.05$ / $p \leq 0.01$)
a)Historical control data range from 1983 – 1989 (6 studies), number of females examined 133, Historical control range 1983-1993 combined from two laboratories (one was performing laboratory)
b)one female had a total litter loss (7 early resorptions) and was excluded from the mean calculations (non pregnant with at least one viable foetus)
c)one foetus with multiple malformations
d)Cheilognathopalatochisis

	## According to public literature from 1969 (Peters and Strassburg, 1969). Not considered appropriate by DK-RMS due to the data being obtained very much outside a 5 year period centered around the B.6.6.2.3.1/01 study as well as the HCD seem to be from mice exposed to noice as a stress factor during gestation. In this study: Control group: one finding of cleft face/jaw/pale (+ other anomalies), 10 mg/kg bw group: four findings of cleft palate (2 in combination with other anomalies), and 100 mg/kg bw group: Six findings of cleft palate (one combined with micrognathia and one finding of each of the following: dilation of brain ventricle, vertebral asymmetry, kinked and shortened tail, partial aplasia of the parietal bone, rib fusion (and floating rib, spinal kink), and 2 finding of spinal dysplasia. However it seems the B.6.6.2.3.1/01 study may be included in the HCD. **HCD in** Table 6.6-59. Examination of the foetuses, could not be verified. HCD for resorptions could not be verified by DK-RMS because some of the documents submitted were scanned handwritten documents, which sometimes were difficult/impossible to read.

b)

Previous evaluation	In DAR (2006) for original approval

Study ID	B.6.6.2.3.1/02
Study title	HWG 1608 – Supplementary study for maternal toxicity on mice following oral administration
Matrix ID	- Not included as no effects in the offspring were examined
Test substance	(HWG 1608) Tebuconazole
Purity (%) **Batch no.**	97.4 16012/86
Test animals	Mated female NMRI/ORIG Kisslegg mice (mated until vaginal plug appeared (day zero of gestation))
Groups	10/dose
Dose	0, 10, 20, 30 or 100 mg/kg bw/day on day 6-15 post mating
Route	Oral by gavage
Vehicle	0.5 % aqueous Cremophor EL solution. Total dosing volume was 5 mL/kg bw
GLP	Yes
Guideline	The method used for dosing relates to "Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation, Human and Domestic Animals", EPA, 83-3, "Teratogenicity Study" from 1984 and OECD guideline 414 (1981). Both the EPA and the OECD guidelines have been revised (OECD guideline 414 in 2001) with extension of the dosing period to cover more than the organogenesis period and a few minor changes
Deviation	There are a number of deviations in relation to the guidelines, which were valid at the time of performing the study. There were few animals in the groups. A large part of the animals were not inseminated. The dams were killed on the 16th day of gestation and no examination of the foetuses was performed. Instead livers, spleens, kidneys and adrenals of half of the animals were weighed and "reduced" clinical chemistry and haematology were performed on 5 females from each dose group. The excised livers were examined histopathologically.
Acceptable	Acceptable as supplementary information on maternal toxicity in mice
NOAEL	N/A – Foetuses were not examined. This was only an investigative study of maternal toxicity in mice. Due to the low number of animals and the low number of pregnancies the study results were not sufficiently reliable to determine a robust maternal LOAEL or NOAEL. However, the top dose of 100 mg/kg bw/day was a clear effect level for maternal toxicity in the mouse.
Effects at the LOAEL	N/A - Decreased body weight gains during dosing, increased liver weights and associated histopathological changes (increased fat content and vacuoles with lipids) were clearly seen at the top dose of 100 mg/kg bw/day.

Methods

Although not a guideline study but an investigative study of maternal toxicity in mice, dosing was performed in

accordance with the EPA Human and Domestic Animals Health Effects Testing Guideline 83 - 3 (1984). Five groups each of 10 inseminated female NMRI/ORIG Kisslegg mice were given daily doses of 0, 10, 20, 30, or 100 mg/kg bw, respectively, of 97.4 % pure tebuconazole in an 0.5 % aqueous Cremophor solution on days 6 - 15 of gestation. All animals were inspected at least once daily with respect to mortality and appearance and behaviour. The dams were killed on the 16th day of gestation and blood was taken from half of them for clinical chemical and haematological testing. The livers were removed from the other half for determination of the weight and histopathological examination. A number of organs were removed for having the weight recorded.

Results

Maternal toxicity

No deaths or clinical signs of toxicity, which could be attributed to dosing, were observed. Mean body weight gains of pregnant animals during the administration time (days 6 – 15) were reduced by - 32 % change compared to control at 100 mg/kg bw/day (Table 6.6-58). While a decrease in body weight gain was also seen at 10 and 20 mg/kg bw/day (> 10 % change compared to control at 20 mg/kg bw/day) a clear dose-response was not seen as body weight gain was increased at the next highest dose of 30 mg/kg bw/day. Liver histopathological changes (cytoplasmic vacuoles containing lipids) were seen at the top dose. Concentrations of triglycerides in the liver were also increased at the top dose (statistically significantly and 164 % change compared to control) (Table 6.6-59). Liver weights in all treatment groups were increased, but not statistically significantly and not in a dose dependent way (Table 6.6-59). Therefore, they were not considered treatment-related. No other organ weights were increased over controls.

Table 6.6-60. Maternal effects

Parameter	**Control**	**10 mg/kg bw**	*(%)[a]*	**20 mg/kg bw**	*(%)[a]*	**30 mg/kg bw**	*(%)[a]*	**100 mg/kg bw**	*(%)[a]*
Number of dams examined	10	10		10		10		10	
Clinical findings during application of test substance	0	0		0		0		0	
Mortality of dams (%)	0	0		0		0		0	
Body weight on last day of dosing [g]	40.0	46.4		43.0		46.0		37.6	
Body weight gain (g) Days of administration (6 – 15) Day 0-end of test (0 – 15)	 12.0 14.2	 10.9 13.7	 *(-9)* *(-4)*	 8.9 11.4	 *(-26)* *(-20)*	 14.8 18.0	 *(+23)* *(+27)*	 **8.2** **10.7**	 ***(-32)*** ***(-25)***
Liver weight (5 animals/dose group) [mg]	1988.2	2430.4	*(+22)*	2368.8	*(+19)*	2547.4	*(+28)*	2379.2	*(+19)*
Pregnancies* (pregnancy rate)	5/10	7/10		6/10		5/10		7/10	

* The low number of pregnancies should be noted

(%)[a] % change compared to control

Table 6.6-61. Triglyceride concentration in liver-homogenates

	Control	**10 mg/kg bw**	**20 mg/kg bw**	**30 mg/kg bw**	**100 mg/kg bw**
Triglyceride content [µmol/g]	11.61	10.30	12.53	11.21	**30.60***
(%)[a]	-	*(-11)*	*(-8)*	*(-3)*	***(+164)***

* Significantly different from study controls *($p \leq 0.05$)*

** Significantly different from study controls *($p \leq 0.01$)*

(%)[a] % change compared to control

A number of changes in some clinical-chemistry and haematological parameters were seen at all dose levels; however, in the absence of a dose-response, none are considered to be treatment-related (Table 6.6-60).

It should be noted however, that these blood and liver results might be have been confounded by the fact that samples from pregnant and non-pregnant animals were analysed together.

Table 6.6-62. Clinical chemistry and haematology parameters

	Control	10 mg/kg bw	20 mg/kg bw	30 mg/kg bw	100 mg/kg bw
Aspartate aminotransferase (AST) [U/L]	262.7	534.6	371.1	562.3*	339.5
Alanine aminotransferase (ALT) [U/L]	46.8	72.2*	59.6	67.3*	60.7
Glutamate-lactate dehydrogenase (GLDH) [U/L]	8.9	15.1	15.2*	12.4	9.9
Total bilirubin (T-BIL) [µmol/L]	1.4	2.4*	1.9*	1.5	1.3
Urea (UREA) [mmol/L]	6.64	8.71*	7.73	6.53	5.87
Cholesterol (CHOL) [mmol/L]	1.61	2.21	2.56*	2.06	1.40
Haematocrit (HK) [L/L]	0.451	0.453	0.432	0.418**	0.425**
Mean cell haemoglobin content (HBE) [pg]	18.64	18.32	17.64*	18.88	18.12
Leucocytes (Leuko) [x10^9/L]	5.96	5.80	5.14	4.58*	5.52
Mean cell volume (MCV) [fL]	54.4	52.6	50.6*	52.2*	51.6**
Mean cell haemoglobin concentration (MCHC) [g/L Ery]	342.8	348.0	348.4	362.2*	352.4
Thrombocyte count (THRO) [x10^9/L]	903.4	893.8	894.8	1087.6*	803.2

* Significantly different from study controls *($p \leq 0.05$)*
** Significantly different from study controls *($p \leq 0.01$)*

Therefore, an effort was made to separate the liver results obtained from pregnant and non-pregnant mice (Table 6.6-61). Absolute and relative liver weights and the degree of fat content were re-calculated in terms of the pregnancy status of each mouse. As a result of this re-calculation, in pregnant mice the mean absolute liver weights were increased (≥ 15 % change compared to pregnant control) from 30 mg/kg bw/day, and mean relative liver weights were increased from 20 mg/kg bw/day. In non-pregnant mice the increase of liver weight started (both absolute and relative) at 100 mg/kg bw/day. In both pregnant and non-pregnant animals, the increase in liver weight was much more noticeable at the top dose of 100 mg/kg bw/day. With regards to the fat content, there was no difference between pregnant and non-pregnant mice; it was slightly increased from 20 mg/kg bw/day, but much more markedly at the top dose (100 mg/kg bw/day).

Table 6.6-63. Liver weight and fat content in pregnant and non-pregnant mice (supplementary analysis)

Parameter		**Control**	**10 mg/kg bw**	*(%)[a]*	**20 mg/kg bw**	*(%)[a]*	**30 mg/kg bw**	*(%)[a]*	**100 mg/kg bw**	*(%)[a]*
Body weight on last day of dosing [g]		40.0	46.4	*(+16)*	43.0	*(+8)*	46.0	*(+15)*	37.6	*(-6)*
Liver – absolute weight [mg]	p	2588	2635	*(+2)*	2880	*(+11)*	3079	*(+19)*	2586	*(±0)*
	np	1588	1612	*(+1)*	1602	*(+1)*	1750	*(+10)*	2070	*(+30)*
Liver – relative weight [mg/100 mg bw]	p	5044	5264	*(+4)*	5803	*(+15)*	5642	*(+12)*	6409	*(+27)*
	np	4915	5038	*(+2)*	4861	*(-1)*	5303	*(+8)*	6160	*(+25)*
Fat content	p	0.0	0.5		1.0		1.0		3.3	
	np	0.7	1.0		1.5		1.5		3.0	

(%)[a] % change compared to control
p pregnant dams (2 – 4 – 3 – 3 – 3 animals at 0 – 10 – 20 – 30 – 100 mg/kg bw/day)
np non-pregnant dams (3 – 1 – 2 – 2 – 2 animals at 0 – 10 – 20 – 30 – 100 mg/kg bw/day)
bw body weight

UK RMS Conclusion
In this non-guideline investigative study of the maternal toxicity of tebuconazole in mice, decreased body weight gains during dosing, increased liver weights and associated histopathological changes (increased fat content and vacuoles with lipids) were clearly seen at the top dose of 100 mg/kg bw/day. Less marked liver effects were seen at lower doses (30 and 20 mg/kg bw/day), but, due to the low number of animals examined, it is difficult to identify a clear NOAEL. However, at the top dose of 100 mg/kg bw/day there was a clear effect level for maternal toxicity in the mouse.

B.6.6.2.3.1b Discussion and conclusion by DK-RMS:	The DK-RMS notes that neither body-, nor liver weight in exposed females were statistically significant different from controls, indicating the observed toxicity at this dose levels was only slight.

c)

Previous evaluation	In DAR (2006) for original approval

Study ID	B.6.6.2.3.1/03
Study title	Combined report of embryotoxicity study (including teratogenicity) and supplementary embryotoxicity study (including teratogenicity) with HWG 1608 (Tebuconazole) in the mouse
Matrix ID	29
Test substance	(HWG 1608) Tebuconazole
Purity (%) Batch no.	96.2 and 95.8 active substance (in RCC 319432) and 96.8 and 96.3 active substance (in RCC 360270) 816196048
Test animals	Mated female NMRI KFM-HAN (outbred, SPF quality) mice (mated until spermatozoa in vaginal smear or vaginal plug were observed)
Groups	Main study: main group 35/dose; subgroup 10/dose Supplementary study: main group 30/dose; subgroup 7/dose
Dose	Main study: 0, 10, 30 or 100 mg/kg bw Supplementary study: 0, 1, 3 mg/kg bw
Route	Oral by gavage
Vehicle	0.5 % Cremophor EL in bidistilled water
GLP	Yes
Guideline	OECD Guideline for the Testing of Chemicals No. 414 ("Teratogenicity") (1981), and EPA, Pesticide Assessment Guidelines 83-3 ("Teratogenicity Study") revised edition (1984). Both the EPA and the OECD guidelines have been revised (OECD guideline 414 in 2001) with extension of the dosing period to cover more than the organogenesis period and a few minor changes
Deviation	Examinations of clinical chemistry, haematology and organ weight and histology do not form an integrated part of the "normal" Teratogenicity Studies. Historic controls are few and vary between the two parts of the study. The following deviations from the OECD-Guideline 414 (2001) occurred: - Dosing was performed during the period of organogenesis only. - Food consumption recorded in five-day intervals instead of three-day intervals.
Acceptable	Acceptable, in a WoE approach.
NOAEL / LOAEL	Maternal NOAEL: 100 mg/kg bw/d. Developmental LOAEL: 10 mg/kg bw/d. Both agreed at PRAPeR Expert Meeting 49 (2-6 June 2008)
Effects at the LOAEL	Maternal: liver effects seen at 30 and 100 mg/kg bw/d were considered adaptive. Developmental: total incidence of malformations (open eye, runts, cleft palate) was increased at the low dose of 10 mg/kg bw/d.

Methods
In a developmental toxicity study in accordance with the OECD Guideline for the Testing of Chemicals No. 414 ("Teratogenicity") (1981), mated female NMRI (KFM-HAN) mice were given, by gavage, once daily, on day 6 through to day 15 of gestation doses of tebuconazole (95.8 - 96.8 % pure).

Animals were inspected for mortality, clinical signs and changes in appearance or behaviour at least twice daily. Body weights were recorded daily and food consumption at regular intervals.

Main study (project no. 319432): The main study consisted of groups of 35 (main groups) and 10 (subgroups) mated female mice. The test substance was given suspended in 0.5 % Cremophor EL solution in doses of 0, 10, 30 or 100 mg/kg bw.

Main groups: This study was to assess the effects of tebuconazole on embryonic and foetal development in pregnant mice. The dams were necropsied on day 18 of gestation. Post mortem examination, including gross macroscopic examination of all internal organs was performed. The adrenals, the kidneys, the liver and the spleen from all gravid dams were weighed separately and prepared for histological examinations, and the gravid uteri were removed by caesarean section and weighed before opening. The following data were recorded in pregnant dams: number of corpora lutea, number of implantation sites, litter size, position, weight and sex of each live foetus, and number of dead foetuses. The foetuses were examined for external abnormalities, and visceral (half of the animals) and skeletal abnormalities and skeletal retardations (the other half of the animals).
Subgroups: This study was to assess the effects of tebuconazole on haematology and clinical biochemistry parameters in mated female mice. Blood specimens were taken from the retro-orbital plexus just prior to sacrifice of the dams on day 16 post coitum and were subjected to full haematological and clinical chemistry analysis. The gravid uteri were removed by caesarean operation and were weighed. All reproduction parameters were recorded (as above). The adrenals, the kidneys, the liver and the spleen from all gravid dams were weighed separately and prepared for histological examinations. Portions were taken from some livers for determination of the cytochrome P-450, the N-demethylase and the O-demethylase activities and the triglyceride content.

Supplementary study (project no. 360270): The supplementary study consisted of groups of 30 (main groups) and 7 (subgroups) mated female mice. The test substance was given suspended in 0.5 % Cremophor EL solution in doses of 0, 1 or 3 mg/kg bw.
Main groups: As above for the main study.
Subgroups: As above for the main study.

Results
Tebuconazole exhibited maternal toxicity in pregnant NMRI mice from 30 mg/kg bw/day. The target organ was the liver (Tables 6.6-62 and 6.6-63.). Although, the dose-response relation is clear at the lower dose only statistically significant findings are considered.

Maternal toxicity
There were no effects on maternal animals at dose levels up to and including 3 mg/kg bw/day (supplementary study). Body weight gains were decreased (13 % change compared to control) at the top dose of 100 mg/kg bw/day (main study) (Table 6.6-62.). Feed intakes were marginally decreased (< 10 % change compared to control) at the top dose of 100 mg/kg bw/day (main study).
The DK-RMS notes that the 13% decrease in maternal body weight gain during treatment at the top dose was not statistically significant, and that no statistically significant effect on body weight was observed at necropsy at this dose.

Effects on the liver were seen at 10 mg/kg bw/d and above. The dose of 10 mg/kg bw/d resulted in enzyme induction (cytochrome P-450, N-demethylase) (Table 6.6-63.) and increased vacuolization of the liver. The liver effects at 10 mg/kg bw/d were minimal and are considered adaptive (< 15 % increase in absolute and relative liver weight and no statistical significance seen for liver enzymes). Higher doses of 30 and 100 mg/kg bw/d resulted in further signs of liver toxicity (increase in liver weight and lipid storage, clinical chemistry and histopathology) (Table 6.6-64.).
The DK-RMS notes that at the dose of 30 mg/kg the effect on liver weight was not statistically significant.

More specifically, liver O-demethylase and plasma alkaline phosphatase were increased at 30 mg/kg bw/d and above (Table 6.6-63.). Transaminases (ASAT and ALAT) were increased at 100 mg/kg bw/d. Overall, adverse effects on the liver were seen from 30 mg/kg bw/d. In addition the top dose showed slight effects on the blood turnover, evident by an increase in reticulocytes combined with an increase in spleen weight (statistically significantly increase and > 10 % change compared to control) (Table 6.6-64.).

Table 6.6-64. Maternal toxicity (project no. 319432 - main study)

Parameter	**Control group 0 mg/kg**	**Low dose 10 mg/kg** *(%)*[a]	**Medium dose 30 mg/kg** *(%)*[a]	**High dose 100 mg/kg** *(%)*[a]
Number of dams examined				
Main groups	35	35	35	35
Subgroup	10	10	10	10

Parameter		**Control group 0 mg/kg**	**Low dose 10 mg/kg** *(%)[a]*		**Medium dose 30 mg/kg** *(%)[a]*		**High dose 100 mg/kg** *(%)[a]*	
Findings during application of test substance		Abortion 1	Crusted sore 1		Mortality 1		--	
Mortality of dams (%)								
	Main groups	-	-		2.9		-	
	Subgroup	10.0	-		-		10.0 (incident)	
Body weight gain [g]	GD 0 – 6	4	4	*(±0)*	4	*(±0)*	3	*(-25)*
	GD 6 – 11	5	5	*(±0)*	5	*(±0)*	5	*(±0)*
	GD 11 – 16	11	12	*(±0)*	11	*(±0)*	**9**	***(-18)***
during treatment	GD 6 – 16							
	Main groups	16	17	*(+6)*	16	*(±0)*	**14**	***(-13)***
	Subgroup	15	16		13		**15**	
Food consumption [g]	GD 0 – 6	7.3	7.5	*(+3)*	7.5	*(+3)*	7.3	*(±0*
	GD 6 – 11	8.7	8.9	*(+2)*	8.4	*(-3)*	8.2	*(-6)*
	GD 11 – 16	9.8	10.1	*(+3)*	9.7	*(-1)*	9.6	*(-2)*
during treatment	GD 6 – 16							
	Main groups	9.3	9.5	*(+2)*	9.1	*(-2)*	8.9	*(-4)*
	Subgroup	9.4	8.9		8.4		9.8	
Pregnancies [%]								
	Main groups	82.9	80.0		68.6		74.3	
	Subgroup	90.0	80.0		50.0		100.0	
Necropsy findings in dams dead before end of test		(No finding)						

GD: gestation day
Data from both main groups and subgroups of the main study are provided.
(%)[a] % change compared to control

Table 6.6-65. Clinical chemistry – main study, subgroups

Parameter (measured in liver homogenate)	**Control group 0 mg/kg**	**Low dose 10 mg/kg**	**Medium dose 30 mg/kg**	**High dose 100 mg/kg**
Number of dams examined	10	10	10	10
Cytochrome P-450 (nmol/g)	29.4	42.3	73.7**	116.4**
(%)[a]	-	*(+44)*	*(+151)*	*(+296)*
N-Demethylase activity (nmol/min/g)	260.4	413.9	752.9**	975.4**
(%)[a]	-	*(+60)*	*(+189)*	*(+275)*
O-Demethylase activity (nmol/min/g)	2.52	2.95	3.67	8.16**
(%)[a]	-	*(+17)*	*(+46)*	*(+224)*
Liver triglyceride (µmol/g)	8.8	7.0	11.9	14.8
(%)[a]	-	*(-21)*	*(+35)*	*(+68)*
ASAT [U/L]	2.74	2.60	2.12	3.17
(%)[a]	-	*(-4)*	*(-22)*	*(+17)*
ALAT [U/L]	1.07	1.38	1.05	1.78
(%)[a]	-	*(+29)*	*(-2)*	*(+66)*
Alkaline phosphatase [U/L]	2.21	2.22	3.77*	3.37
(%)[a]	-	*(+1)*	*(+71)*	*(+53)*

*/** Significantly different from controls *($p \leq 0.05$ / $p \leq 0.01$)*
Data from subgroups of the main study only are provided.
(%)[a] % change compared to control

Table 6.6-66. Necropsy data (both main and supplementary studies)

Parameter	Dose (mg/kg bw/day)						
	0 [a]	**0** [b]	**1** [b]	**3** [b]	**10** [a]	**30** [a]	**100** [a]
Organ weights [g]							
Main groups							
No. of dams examined	35	30	30	30	35	34	35
Body weight	48.1	47.1	48.7 *(+3)*	44.9 *(-5)*	48.7 *(+1)*	45.0 *(-6)*	43.3 ***(-10)***
Liver absolute [g]	2.52	2.48	2.46 *(-1)*	2.33 *(-6)*	2.52 *(±0)*	2.42 *(-4)*	2.78 *(+10)*
Liver (%)[c]	5.29	5.37	5.16 *(-4)*	5.27 *(-2)*	5.23 *(-1)*	5.45 *(+3)*	**6.34**** ***(+20)***
Spleen absolute [g]	0.148	-	-	-	0.154 *(+4)*	0.157 *(+6)*	**0.200**** ***(+35)***
Spleen (%)[c]	0.323	-	-	-	0.337 *(+4)*	0.371 *(+15)*	**0.468**** ***(+45)***
Subgroups							
No. of dams examined	9	7	7	7	10	10	9
Body weight	41.0	47.0	43.0 *(-9)*	45.1 *(-4)*	43.3 *(+6)*	36.1 ***(-12)***	43.8 *(+7)*
Liver absolute [g]	2.22	2.72	2.37* *(-10)*	2.39* *(-7)*	2.42 *(+9)*	2.21 *(±0)*	**3.05**** ***(+37)***
Liver (%)[c]	5.37	5.50	5.30 *(-1)*	5.31 *(-3)*	5.59 *(+4)*	**6.08*** ***(+13)***	**6.94**** ***(+29)***
Spleen absolute [g]	0.168	-	-	-	0.168 *(±0)*	0.163 *(-3)*	**0.213** ***(+27)***
Spleen (%)[c]	0.423	-	-	-	0.391 *(-8)*	0.458 *(+8)*	**0.485** ***(+15)***
Average grade of vacuolisation of the liver (subgroups)							
Liver lipid storage	1.0	-	-	-	2.2	2.2	3.3
Average severity of lipid storage of the liver (subgroups)							
Liver lipid storage	1.8	-	-	-	1.8	2.4	3.5

*/** significantly different from study controls ($p \leq 0.05$ / $p \leq 0.01$)
[a]: main study
[b]: supplementary study
(%)[c] % change compared to control
- not determined

Developmental toxicity

The dose of 10 mg/kg bw/day was tolerated without effects on intrauterine development. The dose of 30 mg/kg bw/day showed an increase of post-implantation loss (> 49 % change compared to control) (Table 6.6-66.). At 100 mg/kg bw/day these findings were more pronounced.

Increased incidences of various malformations and anomalies (exencephaly, open eye, cleft palate, absent or dysplastic vertebrae and abnormal ossification of sternebrae) were seen at 10, 30 and 100 mg/kg bw/day (Table 6.6-67.). These malformations have been re-assessed according to current evaluation criteria and incidences re-calculated by exclusion of dead foetuses, small foetuses without malformations and wart-like growths. The UK-RMS concludes that the incidences of the various malformations at 30 mg/kg bw/day were within historical controls, lacked statistical significance and/or dose-response relationship (Table 6.6-67.). Therefore, a treatment-related effect on the incidence of various malformations was only seen at the top dose of 100 mg/kg bw/day. The PRAPeR Expert Meeting 49 (2-6 June 2008) was of a different opinion (see conclusion).

Table 6.6-67. Intrauterine development (both main and supplementary studies)

Parameter	HCD [1)]	Dose (mg/kg bw/day)						
		0 [a]	**0** [b]	**1** [b]	3 [b]	10 [a]	30 [a]	100 [a]
Corpora lutea / dam	12.0 – 15.6	11.9	12.0	14.1*	12.4	12.5	13.0	12.5
Implantations / dam		11.9	11.0	13.1	11.7	12.5	13.0	12.5

Parameter	HCD [1)]	Dose (mg/kg bw/day)						
		0 [a]	**0** [b]	**1** [b]	3 [b]	10 [a]	30 [a]	100 [a]
Pre-implantation loss (%corp. Lutea)	8.7 – 14.1	0	8.7	7.4	5.8	0	0	0
Post-implantation loss (% impl.)	4.7 – 8.3	8.4	8.3	8.4	11.4	8.0	**12.5**	**35.3***
(%)[c]		-	-				***(+49)***	***(+320)***
Resorptions / dam	0.7 – 1.7	1.0	0.9	1.1	1.3	0.9	1.5	4.3*
Early	0.0 – 1.0	0.8	0.5	0.7	1.1	0.6	1.3	**3.5**
Late	0.4 – 1.6	0.2	0.4	0.4	0.2	0.4	0.3	0.8
Total number of foetuses	1941	316	211	239	186	322	275	213
Total number of litters	177	29	21	20	18	28	24	26
Foetuses / litter	10.0 – 12.8	10.9	10	12	10.3	11.5	11.5	8.2*
Live foetuses / litter		10.9	10	12	10.3	11.5	11.4	8.1
Dead foetuses / litter		0.3	0	0	0	0.3	0.7	0.9
Foetus weight (mean) [g]		1.1	1.3	1.3	1.4	1.1	1.1	1.1*
(%)[c]								
Placenta weight (mean) [g]		0.09	-	-	-	0.09	0.09	0.09
Foetal sex ratio [m/f]		175/141	105/106	124/115	108/78	181/141	147/128	126/87

*/** significantly different from study controls *($p \leq 0.05$ / $p \leq 0.01$)*
[1]: Historical Control Data range from 1983 – 1992 (8 studies, 177 litters, 1941 foetuses)
[a]: main study,
[b]: supplementary study.
(%)[c] % change compared to control

Table 6.6-68. Foetal findings (both main and supplementary studies)

Finding		Historical control data [1]	Dose (mg/kg bw/day)						
			0 [a]	**0** [b]	**1** [b]	**3** [b]	**10** [a]	**30** [a]	**100** [a]
External malformations									
Sum external malformations	F	0.0 – 2.5	0.3	0.5	1.7	1.6	1.3	1.5	10.4#
	L	0.0 – 26.1	3.5	4.8	20.0	16.7	10.7	4.2	46.2#
Exencephaly	F	0.0 – 0.8	0	-	-	-	0.9	0.7	5.2**
	L	0.0 – 9.5	0				10.7	4.2	19.2*
"Open eyes"	F	0.0 – 0.4	0	-	-	-	0.6	0.7	3.3**
	L	0.0 – 5.0	0				7.1	4.2	11.5*
Cleft palate (Palatoschisis)	F	0.0 – 2.00	0.0	0	1.3	0.5	0.3	0.7	3.8**
	L	0.0 – 20.0	0.0	0	15.0	5.6	3.6	4.2	26.9**
	L	(0.0 – 32.0)[2]							
Cleft palate (Palatoschisis)[v]	F		0.7	0	2.6	0.0	0.6	1.6	6.1
	L		3.4	0	15.0	0.0	3.6	8.3	19.2
Hind limb malrotated	F	0.0 – 0.4	-	0.47	-	-	-	-	-
	L	0.0 – 4.8		4.76					
Tail small, bent, curled	F	0.0 – 0.4		0.0	0.4	1.1			0.95
	L	0.0 – 4.8		0.0	5.0	11.1			7.69
Other findings not assessed as malformations									
Number of "runts" (small foetuses <0.6g)			0	0	1-	0	1	2-	3+
	F		0.0	0.0	0.4	0.0	0.3	0.7	1.4
	L		0.0	0.0	5.0	0.0	3.6	4.2	7.7
Warth-like growth on forepaw									
	F		0.3	-	-	-	0.6	1.5	3.3**
	L		3.4				7.1	12.5	15.4

F: % foetuses,
L: %litter,
[a]: main study,
[b]: supplementary study,
[v]: detected at visceral examination only,
[-]: without other malformations,
[+]: with other malformations
[1]: range from 1983 – 1993 (11 studies) for same strain but combined data from 2 labs; one of which is the lab used in the current study
[2]: HCD range from 1983 – 1922 (8 studies) and public literature (Peter & Strassburg, 1969)
[#]: no statistics performed,
*/** significantly different from study controls *($p \leq 0.05$ / $p \leq 0.01$)*

DK RMS Table with body weight calculations

ID	Reference	Species	Body weight (bw) discussion
29	Study B.6.6.2.3.1/03	Mouse	-Doses: 0, 1, 3, 10, 30, 100 mg/kg bw/d (1 and 3 mg/kg bw/d being from a supplementary study with its own control group) - GD 6-15 - No statistically significant effects on maternal bw gain during exp (GD 6-19) in the main study: 16 g (con), 14 g (100 mg/kg bw/d) and food consumption not affected. bw at necropsy was nominally lower in 100 mg/kg bw/d (48.1g in control, 43.3 in 100 mg/kg bw/d), but the difference was not statistically significant.

UK RMS Conclusion
Overall, in this guideline developmental toxicity study in the mouse with additional investigations of maternal toxicity, developmental toxicity (slight increase in post-implantation loss) was seen from a dose of 30 mg/kg bw/day. In addition, at the top dose of 100 mg/kg bw/day the incidence of various external malformations (cleft palate, open eyes, exencephaly, tail abnormalities) was slightly increased. Maternal toxicity also occurred from a dose of 30 mg/kg bw/day. This consisted of liver toxicity (increased liver weight, liver histopathology and associated clinical-chemistry), which was accompanied at the top dose by haematotoxicity and decreases in body weight gains and food consumption. On this basis the UK-RMS considers a NOAEL of 10 mg/kg bw/day can be identified from this study for developmental toxicity and maternal toxicity.

Although the NOAELs above have been identified, the UK-RMS notes that in PRAPeR Expert Meeting 49 (2-6 June 2008) the following NOAELs were agreed:

- The maternal NOAEL was set at 100 mg/kg bw/d: Experts discussed maternal toxicity and noted that liver effects (relative liver weights increased and liver enzymes) were seen at 30 and 100 mg/kg bw/d while no clear effects were found on maternal body weight gain. Since the liver effects were considered by the experts as adaptive but not adverse they set the maternal NOAEL at the highest dose of 100 mg/kg bw/d.
- The developmental LOAEL was set at 10 mg/kg bw/d: It was noted that increased post-implantation loss was observed at 30 mg/kg bw/d (not statistically significant). The total incidence of malformations (open eye, runts, cleft palate) was increased already at the low dose of 10 mg/kg bw/d. After an intense discussion the majority of the experts agreed to set the developmental LOAEL at 10 mg/kg bw/d while several experts were of the opinion that that dose level should be considered as the NOAEL.

The increase in malformations at the low dose of 10 mg/kg bw/day is not clear to the UK-RMS, however, the UK-RMS accepts the decision made at the PRAPeR meeting and the LOAEL of 10 mg/kg bw/day will be taken forward into the risk assessment.

B.6.6.2.3.1b Discussion and conclusion by DK-RMS:	The DK-RMS notes that at the dose of 30 mg/kg neither body- nor liver weight were statistically significantly different from controls, indicating that the observed effects at this dose levels were mild indicating no toxicologically relevance.

	The DK-RMS further notes that the 13% decrease in maternal body weight gain seen at 100 mg/kg bw/day during exposure day 6-15 was not statistically significant, and that no significant effect on body weight was seen at necropsy at this dose. This further indicates that even at the highest dose level the observed maternal toxicity was not marked. DK-RMS agrees with the NOAEL setting and discussion/conclusion from the PRAPeR expert meeting (2008). HCD included in the study report was combined data from the performing laboratory and from another laboratory. HCD or resorptions were not verified by DK-RMS since the data was provided as handwritten scanned documents which were not always readable. HCD from public literature (Peter & Strassburg, 1969) is not considered relevant since the data was collected a very long time before the current study was conducted and was observed under stressful conditions (noise).

B.6.6.2.3.2. Developmental toxicity study following dermal administration to mice

Previous evaluation	In DAR (2006) for original approval

Study ID	B.6.6.2.3.2/01
Study title	Embryotoxicity study (including teratogenicity) with HWG 1608 technical in the mouse (dermal application).
Matrix ID	30
Test substance	(HWG 1608 Technical) Tebuconazole
Purity (%) **Batch no.**	98.1 (main study) and 96.1 (supplementary study A and B). 16002/85 (main study) and 816896061 (supplementary study A and B for additional investigations of maternal toxicity).
Test animals	NMRI KFM- HAN mice (Outbred SPF Quality)
Groups	30-34 mice/dose and a total of 128 mated females (main study). 10 mice/dose/study and a total of 80 mated females (2 supplementary studies (A and B)).
Dose	0, 100, 300 or 1000 mg/kg bw/day on day 6-15 post mating.
Route	Dermal application on 10 % of the body surface, under occlusive dressings for six hours prior to rinsing with lukewarm water.
Vehicle	4 % CMC (carboxymethylcellulose sodium salt) in distilled water. The total volume applied was 2.5 ml/kg bw.
GLP	Yes
Guideline	Pesticide Assessment Guidelines Subdivision F. Hazard Evaluation: Human and Domestic Animals, EPA, § 83-3, Teratogenicity Study, revised edition (1984). Both the EPA and the OECD guidelines have been revised (OECD guideline 414 in 2001) with extension of the dosing period to cover more than the organogenesis period and a few minor changes.
Deviation	Yes, a later numbering of the animals in the raw data due to the necessity to increase the number of animals (to fulfil guidelines with respect to number of litters per group). The following deviations from the OECD-Guideline 414 (2001) occurred: - Dosing was performed during the period of organogenesis only. - Food consumption recorded in five-day intervals instead of three-day intervals.
Acceptable	Acceptable, in a WoE approach.
NOAEL	Maternal : 100 mg/kg bw/day Developmental: 300 mg/kg bw/day
Effects at the LOAEL	Maternal: liver toxicity at 300 mg/kg bw/day Developmental: increased incidence of cleft palate and supernumerary ribs at 1000 mg/kg bw/day (top dose).

Methods
The main study was performed in accordance with OECD guidelines 414 (1984) and EPA guideline 83-3. Groups of 34 (control and mid dose groups) or 30 (low and high dose groups) mated females NMRI KFM-HAN mice were given daily dermal applications of 0, 100, 300 or 1000 mg/kg bw tebuconazole (98.1 % pure) on days 6 to 15 post coitum (p.c.). The substance was dissolved in a 4 % carboxymethylcellulose sodium salt solution and was applied to shaved skin of the back (about 10 % of the body surface) in a volume of 2.5 ml/kg bw. The application was covered with an occlusive bandage and left for 6 hours and was then rinsed off with lukewarm water. The dams were inspected at least twice daily for mortality, clinical signs and changes in appearance and behaviour. Weights of the animals and food consumption were recorded at regular intervals. At day 18 post coitum Caesarean section were performed with the following examinations: gross macroscopic examination of all internal and all external organs, with emphasis on the uterus, uterine contents, position of foetuses in the uterus and number of corpora lutea, was performed and the data recorded. The foetuses were removed from the uterus, sexed weighed individually, examined for gross external abnormalities and allocated to either Wilson's slicing techniques for examination of the viscera and brain – half of the live foetuses. The other half placed in potassium hydroxide solution and stained with alizarin red S and examined for skeleton abnormalities and all abnormalities were recorded. The uteri and contents of all uteri with live foetuses were weighed at necropsy on day 18 post coitum to enable calculation of the corrected body weight gain. If no implantation sites were evident, the uterus was placed in an ammonium sulphide solution.

In the supplementary study groups of 2 x 10 mated females (part A and B) of the above strain were dosed in exactly the same manner as described above. The tebuconazole used was 96.1 % pure. Also observations and weighing of animals and food at regular intervals were performed as in the main study. All dams were sacrificed on day 16 post coitum and necropsied and the liver and adrenals of group A animals were weighed, the pregnancy status of the animals was recorded, and sections of liver and adrenals were examined histopathologically (group A). Before sacrifice of the study B dams, blood samples were collected from non-fasted animals. Following this the females were sacrificed and necropsied. The pregnancy status was recorded and the entire liver was taken for analysis of the cytochrome P-450 content, and the N-demethylase and O-demethylase activities. The blood samples were analysed for aspartate aminotransferase, alanine aminotransferase, glutamate dehydrogenase and alkaline phosphatase.

Results
The main study was a standard teratogenicity study, whereas the supplementary study included additional detailed investigations of maternal toxicity, namely histology of liver and adrenals (A) and clinical chemistry (B), respectively.

Main study
Maternal toxicity
In the maternal animals of the main study, there were no deaths, no systemic clinical signs of toxicity, no local skin reactions and no abnormal findings as well as no effect on feed consumption and mean body weight gain up to the top dose of 1000 mg/kg bw/day (Table 6.6-68).

Table 6.6-69. Maternal effects

Parameter	Control data		Low dose 100 mg/kg	Medium dose 300 mg/kg	High dose 1000 mg/kg
	Historical	Study			
Number of dams examined	25	26	25	26	27
Mortality of dams (%)	0	0	0	0	0
Body weight gain (day 0-end of test)		25	27	28	25
Food consumption (day 6-16)		7.9	8.1	8.1	7.9
Pregnancies (%)	96	96.2	100	92.3	92.6

Developmental toxicity
In general no dose-related effects were observed in the foetuses, but a slightly increased number (not statistically significant) of cleft palate (palatoschisis) and supernumery ribs were found in the foetuses of the 1000 mg/kg bw/day dose group (Tables 6.6-69 & 6.6-70).

Table 6.6-70. Litter responses (Caesarean section data)

Parameter	Control data		Low dose 100 mg/kg	Medium dose 300 mg/kg	High dose 1000 mg/kg
	Historical	Study			
Corpora lutea/number of dams	375/24 = 15.6	359/25 = 14.4	386/25 = 15.4	381/24 = 15.9	382/25 = 13.7
Implantations/number of dams	322/24 = 13.4	317/25 = 12.7	361/25 = 14.4	369/24 = 15.0	303/25 = 12.1
Resorptions/ number of dams [*1]	14/24 = 0.6	16/25 = 0.6	29/25 = 1.2	23/24 = 1.0	18/25 = 0.7
Total number of foetuses	308	301	332	337	285
Pre-implantation loss (%)	14.1	11.7	6.5	5.5	11.4
Post-implantation loss (%) [*1]	4.7	5.0	8.0	6.4	5.9
Total number of litters	24	25	25	24	25
Foetuses / litter	12.8	12.0	13.3	14.0	11.4
Dead foetuses / litter ratio	1/24	0	0	0	0
Foetus weight (mean) (g)	1.2	1.2	1.2	1.1	1.2
Foetal sex ratio (m/f)	156/151	154/147	166/166	186/151	148/137

[*1]: A high number of dams were affected – but only in the low dose group

Table 6.6-71. Examination of the foetuses

Parameter	Control data		Low dose 100 mg/kg	Medium dose 300 mg/kg	High dose 1000 mg/kg
	Historical	Study			
External anomalies[*1] (%)	2.6	3.0	3.0	1.8	**5.3**
Skeletal anomalies[*2] (%)	1.3	2.5	0.6	1.1	1.4
Skeletal variants[*3] (%)	-	-	-	-	-
Visceral anomalies[*4] (%)		3.5	5.6	1.9	**8.6**

[*1]: Control group: Eight findings of palatoschisis (1 with additional tail cranial bended and 1 with additional exencephaly). One finding of malposition of hind leg. 100 mg/kg bw dose group: Eight findings of palatoschisis, one of malposition of hind leg and one exencephaly. 300 mg/kg bw dose group: Four incidents of palatoschisis, one of tail cranial bended and one of malposition of one hind leg. 1000 mg/kg bw dose group: 12 foetuses with palatoschisis (one incl. exencephaly), two foetuses with malpositioned hind leg and one foetus with exencephaly.

[*2]: Control group: Two foetuses with asymmetric sternebrae nos. 4 and 5, one with the same but nos. 2-5 and one foetus with partly missing cranium. 100 mg/kg bw dose group: one finding of asymmetric sternebrae nos. 4 and 5. 300 mg/kg bw dose group: one finding of asymmetric sternebrae nos. 4 and 5, and one supernumery flying rib no. 14. 1000 mg/kg bw dose group: one foetus with asymmetric and bipartite sternebrae nos. 2-5 and one foetus with asymmetric sternebrae nos. 3 and 4.

[*3]: There were a lot of significant differences noted in the skeletal findings (variants) between the control group and all the treated groups based on a foetuses base and on a litters base – but no trends towards and increase with dose could be identified.

[*4]: Control group: Five foetuses with palatoschisis. 100 mg/kg bw dose group: Eight foetuses with palatoschisis and one with exencephaly. 300 mg/kg bw dose group: Three foetuses with palatoschisis. 1000 mg/kg bw dose group; Eleven foetuses with palatoschisis (one with additional exencephaly) and one foetus with exencephaly only.

Table 6.6-72. Examination of the foetuses, detailed effects

Parameter	Tebuconazole [mg/kg bw/day]				HCD[#]	HCD¤
	0	100	300	1000		
Number of fetuses (litters) evaluated	301 (25)	332 (25)	337 (24)	285 (25)	# #	
External examination						
Number of fetuses (litters) affected	9 (8)	10 (8)	6 (6)	15 (9)	- -	
Cleft palate (palatoschisis)	2.7 [a] (24.0)	2.4 (24.0)	1.2 (16.7)	**4.2** [b] (28.0)	0.0 – 2.0 (0.0 – 20.0)	0.0 – 2.0 (0.0 – 20.0)
Malposition of hindlimb	0.3 (4.0)	0.3 (4.0)	0.3 (4.2)	**0.7** **(8.0)**	0.0 – 0.5 (0.0 – 4.8)	0.0 – 0.5 (0.0 – 4.8)
Misshapen tail (small, bent, curled)	0.3 (4.0)		0.3 (4.2)		0.0 – 0.4 (0.0 – 4.8)	
Exencephaly	0.3 [a] (4.0)	0.3 (4.0)	- -	0.7 [b] (4.0)	0.0 – 0.8 (0.0 – 9.5)	0.0 – 0.3 (0.0 – 4.2)
Skeletal examination						
No of foetuses examined	159	171	175	**145**		
Supernumerary rib, one left	58%	62%	60%	**74%****		
Supernumerary rib, one right	48%	58%	52%	**72%****		

#Historical control data (HCD) range from 1983 – 1993 (11 studies, 247 litters, 2760 fetuses), NMRI mice. Combined from two different laboratories in Germany and Switzerland, respectively. One of the laboratories is the performing laboratory.
¤ Historical control data (HCD) range from 1987 – 1993 (5 studies), same mouse strain; NMRI mice. The laboratory is the performing laboratory. No information on breeder.
a One fetus with cleft palate and additional tail cranial bended; another fetus with cleft palate and additional exencephaly.
b One fetus with cleft palate and additional exencephaly.
c Visceral examination: no further findings were observed in any treatment group compared to findings during external examination. Findings considered related to treatment with tebuconazole are written in bold letters.
** $p \leq 0.01$

Supplementary study

Maternal toxicity

In the supplementary study, absolute adrenal weight was decreased at all dose levels (statistically significant and > 10 % change compared to control, no clear dose-response), a reduction in relative adrenal weight was also seen (>10 % change compared to control with a dose-response), however, statistical significance was only observed at the top dose (Table 6.6-72.). In the absence of associated histopathology, these decreases are not considered adverse. Liver toxicity was observed from 300 mg/kg bw/day. Histological examination showed hepatic fatty change of periportal areas in all mice in the 1000 mg/kg bw/day dose group and in most mice in the 300 mg/kg bw/day dose group. Clinical biochemistry investigation on blood and liver tissue samples showed a

statistically significant increase in ALT activity (38 % change compared to control) (1000 mg/kg bw/day dose group) and in cytochrome P-450 content, N-demethylase and O-demethylase activity (> 15 % change compared to control at 300 and 1000 mg/kg bw/day dose groups, no clear dose-response) (Table 6.6-71.).

Table 6.6-73. Maternal effects – supplementary study (clinical biochemistry)

Parameter	Control data		Low dose 100 mg/kg	Medium dose 300 mg/kg	High dose 1000 mg/kg
	Historical	Study			
Number of dams examined [N]		9	10	10	10
Aspartate aminotransferase (µkat/L)		1.72	2.25	2.28	2.36
Alanine aminotransferase (µkat/L) *(%)[a]*		0.80 -	0.88 *(+10)*	0.96 *(+20)*	**1.10*** ***(+38)***
Glutamate dehydrogenase (nkat/L) *(%)[a]*		220.5 -	277.8 *(+26)*	476.2 *(+116)*	407.9 *(+85)*
Alkaline phosphatase (µkat/L) *(%)[a]*		1.99 -	2.02 *(+1.5)*	2.67 *(+34)*	1.99 *(±0)*
Cytochrome P-450 (nmol/g) *(%)[a]*		30 -	45.1 *(+50)*	**74.2**** ***(+147)***	**73.6**** ***(+145)***
N-demethylase (nmol-HCHO/min/g) *(%)[a]*		331 -	394.5 *(+19)*	**691.9**** ***(+109)***	**640.5**** ***(+94)***
O-demethylase (nmol/min/g) *(%)[a]*		32.27 -	31.40 *(+19)*	**43.78**** ***(+19)***	**41.09**** ***(+19)***

* Significantly different from study controls *(p ≤ 0.05)*
** Significantly different from study controls *(p ≤ 0.01)*
(%)[a] percent change compared to control.
N: number of individuals

Table 6.6-74. **Maternal effects – supplementary study (organ weight)**

Parameter	Study Control	Low dose 100 mg/kg	Medium dose 300 mg/kg	High dose 1000 mg/kg
Number of dams examined	10	10	10	10
Body weight (g)	42.6	39.7	41.9	47.4
Liver (g) - absolute *(%)[a]*	2.39 -	2.25 *(-6)*	2.38 *(±0)*	2.73 *(+14)*
Liver (%) - relative *(%)[a]*	5.60 -	5.63 *(+1)*	5.67 *(+1)*	5.75 *(+3)*
Adrenals (g) - absolute *(%)[a]*	0.017 -	**0.012*** ***(-29)***	**0.011**** ***(-35)***	**0.012*** ***(-29)***
Adrenals (%)- relative *(%)[a]*	0.040 -	**0.033** ***(-18)***	**0.029** ***(-28)***	**0.025*** ***(-38)***

* Significantly different from study controls *(p ≤ 0.05)*
** Significantly different from study controls *(p ≤ 0.01)*
(%)[a] percent change compared to control.

UK-RMS Conclusion

In a guideline dermal developmental toxicity study in mice with additional detailed investigations of maternal toxicity, slightly increased incidences of cleft palate and supernumerary ribs was seen at the top dose of 1000 mg/kg bw/day. Maternal toxicity consisting of liver toxicity (fatty changes and induction of mixed-function oxidase activities) was observed from the mid-dose of 300 mg/kg bw/day. On this basis, a NOAEL for maternal toxicity of 100 mg/kg bw/day and a developmental NOAEL of 300 mg/kg bw/day were identified. It is noted that the maternal NOAEL is the value agreed during the first review of tebuconazole. However, the developmental NOAEL has been lowered from 1000 to 300 mg/kg bw/day.

B.6.6.2.3.1b Discussion and conclusion by RMS-DK:	The DK-RMS notes that at doses of both 300 mg/kg bw/day and 1000 mg/kg bw/day neither maternal body weight nor maternal liver weights were significantly changed from controls, indicating that the observed maternal toxicity at these doses were mild after dermal exposure. The DK-RMS further notes that a dermal dose of 1000 mg/kg bw/day most likely results in very different internal concentrations of the active compound than oral dosing, due to ADME differences related to exposure route. In spite of the mild signs of maternal toxicity indications of developmental toxicity effects were observed at the top dose. The HCD referenced seems to be from the study report for B.6.6.2.3.1/03. The HCD are combined from the performing laboratory in Switzerland and a laboratory in Germany. HCD is from NMRI mice. HCD only from performing laboratory is also shown in table Table 6.6-75. These data are from the performing laboratory only, from 5 studies and from the same mouse strain. Information on breeder was not available. % pregnancies could not be verified, though.

B.6.6.2.3.3. UK-RMS Summary of mice developmental studies

The potential for tebuconazole to adversely affect development in the mouse was investigated in four guideline studies; three by oral administration (one of which was limited to an investigative study of maternal toxicity) and one by dermal administration.

In the oral studies, developmental toxicity (runts, increased incidence of post-implantation loss and delayed ossification) started to occur from 30 mg/kg bw/d, becoming more severe (reduced foetal weight and slightly increased incidence of external malformations such as cleft palate, exencephaly, malrotated hind limb and tail abnormalities) at 100 mg/kg bw/d. Therefore, an overall NOAEL of 10 mg/kg bw/d was identified for developmental toxicity in the mouse. Maternal toxicity (liver toxicity) also started to occur from 30 mg/kg bw/d, becoming more severe (haematotoxicity, decreased body weight gain, reduced food consumption) at 100 mg/kg bw/d. Therefore, an overall NOAEL of 10 mg/kg bw/d was also identified for maternal toxicity in the mouse.

In the dermal study, increased incidences of cleft palate and supernumerary ribs were seen at top dose of 1000 mg/kg bw/d, at which maternal toxicity (liver toxicity) also occurred. Therefore, a NOAEL of 300 mg/kg bw/d was identified for both developmental and maternal toxicity from this study.

It is possible that some of the developmental effects observed in the mouse were the secondary unspecific consequence of maternal toxicity.

B.6.6.2.3.3. Overall summary by DK-RMS:	In the oral studies, developmental toxicity (small foetuses (runts), increased incidence of post-implantation loss and delayed ossification) started to occur from 30 mg/kg bw/d (B.6.6.2.3.1/03), becoming more severe (reduced foetal weight, reduced litter size and slightly increased incidence of external malformations such as cleft palate, exencephaly and tail abnormalities) at 100 mg/kg bw/d (B.6.6.2.3.1/03, B.6.6.2.2.1/04, B.6.6.2.3.1/01). Cleft palate was seen in two mouse studies. In one study, the number of foetuses with cleft palate was increased (> 10 % change compared to control) (B.6.6.2.3.1/01). Cleft palate is a common malformation in this strain of mice, however incidence at 100 mg/kg bw/day were outside of the range of the HCD provided. Other malformations and anomalies (face malformations, kinked and shortened tail, dilation of brain ventricles, vertebral asymmetry, spinal dysplasia, rib fusion, partial aplasia of parietal bone) were also increased above controls and historical control data (> 10 % change compared to control at 100 mg/kg bw/day). In another study (TG 414), the total incidence of malformations (open eye, runts, cleft palate) was increased already at the low dose of 10 mg/kg bw/d (B.6.6.2.3.1/03). In a dermal study, an increased incidence of cleft palate and supernumerary ribs was seen at 1000 mg/kg bw/day (top dose) associated with liver effects in the dam (B.6.6.2.3.2/01).

	Maternal toxicity (liver toxicity) also started to occur from 30 mg/kg bw/d, becoming more severe (haematotoxicity, decreased body weight gain, reduced food consumption) at 100 mg/kg bw/d. Therefore, the UK RMS considers an overall NOAEL of 10 mg/kg bw/d can be identified for maternal toxicity in the mouse. The UK-RMS notes that in PRAPeR Expert Meeting 49 (2-6 June 2008) the following NOAELs were agreed: the maternal NOAEL was set at 100 mg/kg bw/d – since liver effects were considered by the experts as adaptive but not adverse, and the developmental LOAEL was set at 10 mg/kg bw/d – based on an increased incidence of malformations (open eye, runts, cleft palate) in B.6.6.2.3.1/03. The UK RMS accepts the decision made at the PRAPeR meeting and the LOAEL of 10 mg/kg bw/d will be taken forward. The DK RMS agrees with this NOAEL for maternal effects and LOAEL for developmental effects, however noting that effects on maternal body weight gain and altered liver weight and histology in pregnancy may be a direct and specific effect related to an endocrine mode of action, rather than an unspecific secondary effect of maternal toxicity. The DK-RMS further notes that in dermal studies some indication of liver toxicity may have occurred, but in most cases liver weights were not significantly affected and neither were terminal body weight in the exposed females. The dermal exposure most likely results in very different internal concentrations of the active compound than oral dosing, due to ADME differences related to exposure route. In spite of the mild signs of maternal toxicity seen in the studies with dermal exposure, some indications of developmental toxicity effects were observed. Thus, the observed developmental effects in mice cannot be consided related to unspecific maternal toxicity. Further discussion on possible relations with maternal effects is presented below.

B.6.6.2.3.4. Evaluation of applicant's case for dropping the mouse model for developmental toxicity

The Bayer Task Force (BTF) submitted a case under the heading *'Rationale why the mouse is not a recommended species for developmental toxicity studies, and that the mouse model has been generally dropped due to the high background noise on malformations'*. The UK-RMS has evaluated the applicant's case and a summary is provided below together with the opinion of the UK-RMS:

A summary of the case presented

The applicant first pointed the reader towards the developmental toxicity test guidelines:

- OECD test guideline number 414 (1981) states: *"Species commonly used are the rat, mouse, hamster and rabbit. The preferred species are the rat and the rabbit."*
- However, in later guideline versions (OECD test guideline number 414 (2001)), the mouse is no longer mentioned as a test species of choice for developmental toxicity studies - *"The preferred rodent species is the rat and the preferred non-rodent species is the rabbit. Justification should be provided if another species is used."*

The applicant is of the opinion that the guideline changes reflect the scientific progress made in understanding that the mouse is not a suitable test species for the assessment of a specific teratogenic potential of chemicals.

Hayes (1994) noted that minor visceral malformations are 80 % more common in mice compared to rats (3.68 % versus 2.02 %), and minor skeletal malformations are almost twice as likely in mice as compared to rats (5.32 % versus 2.35 %) (Hayes, 1994). The high background variation is further highlighted by the number of litters required to detect a 5 % change in foetal body weight. To detect such a change in CD rats, 62 litters would be required. For CD1 mice, 84 litters, and for C57BL/6 mice, 198 litters are required (Hayes, 1994).

The BTF is of the view that a good scientific approach to study the high "background noise" of malformations in mice is to examine the effect of unspecific stressors to pregnant mice on the development of malformations in their offspring. Two respective publications that discuss the effects of maternal stress on teratology endpoints were cited.

1) Peters and Strassburg (1969) stressed pregnant mice in various ways and summarise their results as follows:

"Experimental study on cleft palate production in animals
In mice, isolated malformations of the palate of different degree were produced experimentally.
For exogenous stimuli before and during the phenocritical phase of palate closure, the following measures served:
1) production of an immunologic shock (booster effect) by a single subcutaneous re-injection of a foreign serum after animals were made sensitive;
2) exclusive feeding chemically non-treated raisins of different vines for a period of 24 h;
3) single withdrawal of solid food for 10 h with normal water supply ad libitum;
4) repeated exposure to noise for 1 h at a time during the day with intervals of rest.
Compared with the control animals each test method produced a significantly higher rate of malformations. The results permit the conclusion that during the phenocritical phase of palate closure obviously any unphysiologic exogenous stimulation may show teratogenic effects provided it has previously produced a stress-situation in the mother animals."

2) Golub *et al.* (2004) discuss the differences between mice and rats in their susceptibility – and therefore suitability as a useful test model – to non-chemical stress factors:

"In mice, an increased incidence of cleft palate, exencephaly, supernumerary ribs, fused ribs, and resorption can be produced by restraint procedures, depending on the timing, and type of restraint, and the strain of mouse. ... Limited research indicates that restraint does not lead to cleft palate or other gross malformation (exencephaly, microphthalmia) in rats ... . Cleft palate induction in restrained mice was as high as 69 % of foetuses as compared to 1 % in controls...)."

The suitability of mice for developmental toxicity study was also considered in the tebuconazole evaluation by the 2010 JMPR meeting (JMPR evaluations 2010, Part II – Toxicological):

1) The expert opinion of Christian (2005) contains a discussion of the evolution of the current teratology guidelines and a history of the use of mice in regulatory toxicology studies. Christian (2005) discusses why the mouse was initially used in teratology studies in the 1950's and why it is now no longer a recommended species for regulatory teratology studies:

1) small size being disadvantageous because it makes examination of foetuses difficult and often prevents obtaining adequate sample sizes in studies requiring blood or tissues;
2) breeding is sometimes erratic;
3) several strains have high and variable rates of background malformations and intra-uterine death.

Christian (2005) also discusses the NMRI strain of mice in particular and points out that NMRI mice are not considered a suitable strain for teratology tests as:

1) there does not exist an adequate historical control database;
2) this strain has a high incidence of spontaneous malformations;
3) this strain has a high rate of spontaneous genetic defects;
4) this strain has a high reactivity to environmental stress.

Christian (2005) concluded that *"observations of malformations in the foetuses of NMRI mice should not be considered an appropriate criterion for calculation of a NOEL"*.

2) Similarly, the suitability of the mouse as a test species for developmental toxicity studies is addressed by Neubert (2000). He stated that *"It should be noted that the mouse as a species has a strong tendency to display unspecific reactions in response to 'stress' (e.g. hunger, restraint, etc.). It is therefore not used routinely in prenatal toxicology, but only for special investigations."*

The BTF concluded that it is now generally accepted that the mouse is not an appropriate species for the assessment of a possible specific teratogenic potential of chemicals and it is, therefore, no longer quoted as an appropriate test species in the current OECD and OPPTS teratology test guidelines.

Opinion of the UK RMS:
The UK-RMS agrees that the mouse developmental studies should be interpreted with caution; however, the UK-RMS disagrees that these studies should be disregarded completely. The UK-RMS notes that these studies show

similar effects to those seen in rats and rabbits at similar dose levels.

B.6.6.2.3.4 Discussion and conclusion by DK-RMS:	The DK-RMS agrees with the conclusion of UK-RMS.

B.6.6.3 Publications of relevance to reproductive toxicity

Eight publications of potential relevance to reproductive toxicity have been considered.

1)

Previous evaluation	None – publication submitted for the purpose of renewal

Study ID	B.6.6.3/01
Author(s)	Dreisig *et al.* (2013)
Study title and journal	Predictive value of cell assays for developmental toxicity and embryotoxicity of conazole fungicides. Altex, Vol 30, 3, 319-330
Matrix ID	59
Test substance	Tebuconazole (and other conazoles)
Purity (%) **Batch no.**	98 EHRC17178700
GLP	No
Guideline	No
Deviation	n/a
Reliability	Reliable with restrictions
Relevance to hazard assessment	Limited relevance as paper describes *in vitro* investigations only.

Methods
In this publication, tebuconazole (and other 4 conazoles) was tested in an embryonic stem cell test (EST). Two stable mouse cell lines were used: 1) an embryonic stem (ES) cell clone D3 to represent undifferentiated embryonic tissue and 2) 3T3 fibroblasts to represent differentiated adult tissue. Concentrations of tebuconazole ranging from 7.8 to 500 µM were used and cytotoxicity measured by applying the resazurin assay. The cytotoxicity data were used to assess if stem cells are more sensitive to toxic agents than adult cells by determining the inhibition of growth of D3 and 3T3 cells. Three single endpoint values (50 % inhibition of cardiac cell differentiation (ID_{50}), 50 % viability of D3 cells (IC_{50}D3), and 50 % viability of 3T3 cells (IC_{50}3T3)) for differentiation and cytotoxicity were determined.

Results and conclusions
IC_{50} values for resazurin reduction were in the range of approximately 10 - 76 µM and 25 - 132 µM for D3 and 3T3 cells, respectively, and 50 % inhibition of cardiac beating was found in the range of approximately 15 – 69 µM. All five conazoles were classified as “weakly embryotoxic” when using this assay.

Table 6.6-76. Embryonic Stem Cell Test endpoints for five conazole compounds

Conazole fungicide	***In vitro* EST assay endpoints**					
	ID_{50} µM (95 % C.I.)	**IC_{50}D3 µM (95 % C.I.)**	**IC_{50}3T3 µM (95 % C.I.)**	**EST classification**	**ID_{50}/IC_{50}D3 ratio**	**IC_{50}D3/IC_{50}3T3 ratio**
Epoxiconazole	33.8 (25.6-	69.4 (51.3-	97.0 (40.1-	Weakly	0.5[a]	0.7

	44.5)	94.0)	234.6)	embryotoxic		
Ketoconazole	15.4 (14.1-16.9)	9.9 (7.0-14.1)	25.6 (20.3-32.2)	Weakly embryotoxic	1.6	0.4[a]
Prochloraz	36.5 (24.6-54.2)	46.7 (38.7-56.5)	60.1 (42.4-87.2)	Weakly embryotoxic	0.8	0.8
Propiconazole	46.3 (31.8-67.3)	76.1 (62.0-93.4)	116.8 (69.7-195.5)	Weakly embryotoxic	0.6	0.7
Tebuconazole	69.1 (44.4-107.4)	74.7 (44.0-126.6)	131.9 (87.1-199.9)	Weakly embryotoxic	0.9	0.6

EST endpoints and their 95% confidence intervals (95% C.I.) were derived from the non-linear regressions to the plots;
a = ID50 and/or IC50 values were considered different as their 95% C.I. were not overlapping.

The UK RMS deems that, given the availability of *in vivo* developmental studies, these *in vitro* findings are of limited relevance to the hazard assessment of tebuconazole.

(Dreisig *et al.*, 2013)

B.6.6.3/01 Discussion and conclusion by DK-RMS:	The DK-RMS agrees with this conclusion.

2)

Previous evaluation	None – publication submitted for the purpose of renewal

Study ID	B.6.6.3/01
Author(s)	Di Renzo *et al.* (2011)
Study title and journal	Is the amphibian X. laevis WEC a good alternative method to rodent WEC teratogenicity assay? The example of the three triazole derivative fungicides Triadimefon, Tebuconazole, Cyproconazole. Reproductive Toxicology, 32(2), 220-226
Test substance	Tebuconazole (and other conazoles)
Purity (%) **Batch no.**	Not specified Not specified
GLP	No
Guideline	No
Deviation	n/a
RMS UK Reliability	Not reliable – no information on purity and number of embryo used; also other reporting deficiencies
DK RMS Reliability	Acceptable for WoE
Relevance to hazard assessment	Limited relevance as paper describes *in vitro* investigations only. Only the rat embryo investigations are described below.

Methods

9.5 day old rat embryos were cultured *in vitro* for 48 hours with a range (15.6 - 250 µM) of tebuconazole concentrations. Following treatment, the embryos were analysed for the appearance of malformations.

Results and conclusions

At the exposure levels of 62.5 - 250 µM tebuconazole induced branchial arches malformations (first and second branchial arches partially or totally fused with unseparated ectomesenchymal areas) in a concentration-related manner. The concentration of 31.25 µM was without effect.

The UK RMS deems that, given the availability of *in vivo* developmental studies, these *in vitro* findings are of limited relevance to the hazard assessment of tebuconazole.

(Di Renzo *et al.,* 2011)

B.6.6.3/02 Discussion and conclusion by DK-RMS:	RMS-DK deems that these effects are relevant for assessment of embryotoxicity as they demonstrate in vitro what is also shown in vivo: that tebuconazole induces teratogenicity similar to other disruptors of retinoic acid signalling. Due to the nature and quality of the study (purity not stated and other shortcomings in reporting it can only be used in a WoE approach).

3)

Previous evaluation	None – publication submitted for the purpose of renewal

Study ID	B.6.6.3/03
Author(s)	Zhou *et al.* (2016)
Study title and journal	Triazole fungicide tebuconazole disrupts human placental trophoblast cell functions. Journal of Hazardous Materials, 308, 294-302.
Matrix ID	60
Test substance	Tebuconazole
Purity (%) **Batch no.**	Not specified Not specified
GLP	No
Guideline	No
Deviation	n/a
Reliability	Not reliable – no information on purity; also other reporting deficiencies
Relevance to hazard assessment	Limited relevance as paper describes *in vitro* investigations only.

Methods

The human placental trophoblast cell line HTR-8 was treated *in vitro* with tebuconazole at concentrations ranging from 5 to 80 µM for up to 72 hours. Following treatment, cell viability (including apoptosis), cell cycle progression, cell migration and the expression of specific genes involved in the modulation of trophoblast functions were investigated.

Results and conclusions

Tebuconazole reduced cell viability, disturbed normal cell cycle progression and induced apoptosis of this cell line. The results demonstrated that tebuconazole induced apoptosis of trophoblast cells via mitochondrial pathway. The invasive and migratory capacities of HTR-8 cells decreased significantly after tebuconazole treatment. In addition, tebuconazole altered the expression of key regulatory genes involved in the modulation of trophoblast functions.

The UK RMS deems that, given the availability of *in vivo* developmental studies, these *in vitro* findings are of limited relevance to the hazard assessment of tebuconazole.

(Zhou et al., 2016)

4)

Previous evaluation	None – publication submitted for the purpose of renewal

Study ID	B.6.6.3/04
Author(s)	Hass *et al.* (2012)
Study title and	Adverse effects on sexual development in rat offspring after low dose exposure to a

journal	mixture of endocrine disrupting pesticides. Reproductive Toxicology, 34(2), 261-274.
Matrix ID	52
Test substance	Tebuconazole (and other pesticides)
Purity (%) **Batch no.**	98.5 Not specified
GLP	No
Guideline	No
Deviation	n/a
UK-RMS Reliability	Reliable with restrictions – low number of exposed dams; high variability; reporting shortcomings; lack of maternal toxicity (reported in other studies); inconsistent findings with those of subsequent studies performed by the same research group.
DK-RMS reliability	Reliable with restrictions – group size 6-8 exposed and 15 controls. Notes: DK-RMS does not find the lack of maternal toxicity at 50 mg/kg unexpected, it is consistent with several other studies. Furthermore, DK-RMS does not find the results presented here to be inconsistent with other studies by the same research group, on the contrary they all point in the same direction. Please refer to "Conclusion" for further details.
Relevance to hazard assessment	Relevant with restrictions

Methods
Pregnant Wistar female rats (10 - 12/dose group) were treated by oral gavage with 12.5 or 50 mg/kg bw/d tebuconazole (in corn oil) from GD 7 to PND 16.

Table 6.6-77. Dose and group size

Dose [mg/kg bw/day]	Number of treated rats	Number of dams with viable litters
0	22	15
12.5	12	8
50	10	6

The pups were checked for genital malformations, nipple retention and ano-genital distance (AGD). In addition, tebuconazole was tested in three *in vitro* endocrine activity tests (androgen receptor reporter assay in CHO cells; T-screen in GH3 cells and steroid synthesis in H295R cell line).

Results and conclusions
Developmental toxicity study
There were no statistically significant effects on maternal body weight gain from GD 7 to GD 21 or from GD 7 to PD 1 in exposed dams compared to controls. No clinical signs of toxicity were observed in the dams and the number of implantation scars in the uterus, post implantation and perinatal loss was similar among groups. Gestation length was not affected by tebuconazole treatment.

Pup body weights in the exposed groups were lower when measured at birth and on PND 6, 13 and 22, but the differences were not statistically significant from controls. The results of the scoring of external genital malformations in male offspring treated with tebuconazole alone sacrificed on PND 16 and 22 and alive shortly after sexual maturation (around PND50) showed no statistically significant differences compared to control.

On PND13, nipple retention in male offspring, an indication of antiandrogenic activity, was significantly increased in the high dose tebuconazole group (1.6 areolas vs 0 in controls). No statistically significant changes on anogenital distance (AGD) on AGD index (AGDI = AGD/cubic root of body weight) were seen in the male offspring on PND1. In the PND1 female offspring, an increase in AGD and AGDI was seen from 12.5 mg/kg bw/d, but no clear dose-response was apparent.

Table 6.6-78. Offspring data. Data represent group means based on litter means±SD.

Offspring (data from viable litters)	1: Control	11: Tebu-12.5	12: Tebu-50
Male birth weight (g)	6.5 ± 0.4	6.3 ± 0.4	6.1 ± 0.4
Female birth weight (g)	6.1 ± 0.4	6.0 ± 0.3	5.9 ± 0.2
Male AGD (units)	24.5 ± 1.1	24.7 ± 1.3	24.6 ± 0.8
Male AGD/cubic root bw (AGDI)	13.1 ± 0.5	13.4 ± 0.7	13.5 ± 0.4
Female AGD (units)	13.6 ± 0.6	14.4 ± 1.0	14.5 ± 0.5 *
Fem. AGD/cubic root bw (AGDI)	7.4 ± 0.3	7.9 ± 0.6 *	8.0 ± 0.2 **
No. Areolas males[a]	0.0 ± 0.0	0.5 ± 0.8	1.6 ± 0.4***
No. Areolas. females	12.0 ± 0.4	12.5 ± 0.6	12.2 ± 0.2
Male body weight PD 6 (g)	12.9 ± 1.3	12.1 ± 1.7	12.3 ± 1.2
Female body weight PD 6 (g)	12.5 ± 1.2	11.9 ± 1.7	12.1 ± 1.2
Male body weight PD 13 (g)	26.1 ± 4.1	24.2 ± 2.7	24.8 ± 2.0
Fem. body weight PD 13 (g)	25.4 ± 3.9	23.9 ± 2.1	24.4 ± 2.4
Male body weight PD 22 (g)	45.6 ± 6.7	43.0 ± 4.6	43.8 ± 4.5
Fem. body weight PD 22 (g)	45.2 ± 6.5	42.7 ± 3.4	42.8 ± 4.5

In vitro endocrine activity assays

Tebuconazole showed androgen receptor antagonism *in vitro.* The lowest observed effect concentration was 3.8 µM. Tebuconazole showed an antagonistic effect in the T-screen (reducing the action of T3) and reduced testosterone and estradiol concentrations whilst increasing progesterone concentration in the H295R steroid synthesis assay.

Overall, tebuconazole showed endocrine activity in a number of *in vitro* tests, affecting steroidogenesis, causing inhibition of the androgen receptor and reducing the action of T3. The UK RMS doubts the reliability of these findings as it is questionable that the substance is capable of affecting multiple targets and it is most likely these were unspecific responses. In addition some of these results are inconsistent with those of other studies.

UK-RMS Conclusion

AGD was increased in females on PND1 from the lowest dose tested of 15 mg/kg bw/d (although no dose-response was apparent) and nipple retention was increased in males on PND13 at the top dose of 50 mg/kg bw/d. No maternal toxicity was observed. The UK RMS notes that the absence of maternal toxicity at the top dose of 50 mg/kg bw/d is inconsistent with the findings of several regulatory developmental toxicity studies, bringing into question the reliability of the reported findings.

The UK RMS also notes that despite the reported effects on AGD on PND1 in females from 15 mg/kg bw/d (although no clear dose-response observed), there were no effects on onset of puberty and mating behaviour up to 50 mg/kg bw/d in subsequent studies performed by the same research group (Jacobsen *et al.,* 2013; Overgaard *et al.,* 2013; see below). It is also noted that an effect on AGD on PND1 was not reported at 50 mg/kg bw/d (but only at 100 mg/kg bw/d) by Taxvig *et al.* (2007- see below). The UK RMS notes that Taxvig *et al.* (2007) reported an effect on AGD on GD21 from 50 mg/kg bw/d, but the same effect on AGD on GD21 was not reproduced at 50 mg/kg bw/d (the only dose tested) by the same authors (Taxvig *et al.,* 2008) in a subsequent study. Therefore, the reported findings on AGD are either not treatment-related or of no toxicological significance.

The increase in nipple retention (1.6 areolae vs 0 in controls) in males on PND13 at 50 mg/kg bw/d has been confirmed in another study by the same research group (Taxvig *et al.,* 2007; see below) from 50 mg/kg bw/d, but no dose-response was noted (3.43 and 3.07 areolae at 50 and 100 mg/kg bw/d vs 2.08 areolae in controls). The UK RMS notes that in this study, the higher number of areolae seen at 50 mg/kg bw/d (1.6) was even lower than the number of areolae in control male pups in the Taxvig *et al.* (2007) study (2.08). Therefore, the UK RMS believes that the claimed effect on nipple retention in PND13 male pups is not treatment-related. This is further supported by the absence of effects on mammary gland development in male pups at PND22 and PND50 in the study by Jacobsen *et al.* (2013 – see below) from the same research group. Therefore, the UK RMS proposes that a NOAEL for developmental toxicity of 50 mg/kg bw/d should be identified from this study in rats. Such NOAEL is consistent with the overall developmental NOAEL of 30 mg/kg bw/d for the rat and 10 mg/kg bw/d for the rabbit and mouse identified from the regulatory studies.

(Hass *et al.,* 2012)

B.6.6.3/04 Discussion and conclusion by DK-RMS:	DK-RMS does not agree with UK-RMS with respect to the conclusion on the endocrine activity *in vitro*, DK-RMS considers it plausible that a chemical substance can have multiple specific modes of action. This has been shown for many different chemical classes. Furthermore other studies have also reported tebuconazole to be an androgen receptor antagonist and disrupts steroidogenesis. DK-RMS notes that a lack of effect on maternal body weight at 50 mg/kg bw/d is not conflicting with evidence from other oral *in vivo* studies in rats. Dosing regimen should be considered, as a bolus dose given by gavage could affect the animals somewhat differently than continued exposure through the feed (used in both the 2-generation reproductive toxicity study and developmental neurotoxicity studies of tebuconazole). The Guideline studies have not investigated a dose level of 50 mg/kg bw/d. There were some effects at 60 mg/kg bw/d on bw in the OECD 414 compliant study by Becker et al., 1988a and more at 120 mg/kg bw/d but that does not imply that there were no effects on bw at 50 mg/kg bw/d. It is likely that 50-60 mg/kg bw/d may be the threshold for maternal toxicity to occur. Thus, chance or specific study details may determine if toxicity occur in a specific study or not. Tebuconazole caused increases in female AGD and AGDI on PND1. For AGD at 50 mg/kg bw/d and AGDI from 12.5 mg/kg bw/d, this can be seen as a dose-response pattern. The actual numbers are only slightly different between the two doses. This phenomenon of a plateaued dose-response curve is quite normal for female AGD and the distance should not be expected to be able to increase indefinitely. Additionally, as AGD is a more sensitive endpoint it cannot necessarily be expected that effects on AGD are followed up with subsequent changes to puberty and/or mating behaviour later in life. The lack of effects on these endpoint later in life cannot be used to conclude that effects on AGD is of no toxicological relevance. DK-RMS notes that the effects on female AGD can be summarized as follows: - Hass et al., 2012 finds increased female AGDI on PND1 at 12.5 mg/kg bw/d and 50 mg/kg bw/d, - Taxvig *et al* 2007 find a increase at 100 mg/kg bw/d on PND1 and on GD21 at both 50 and 100 mg/kg bw/d. - Taxvig et al., 2008 observed no effects at 50 mg/kg bw/d at GD21. Thus the 2 studies examining female AGD on PND1 report effects. Furthermore, already at GD21 there is one study reporting effects while another did not. It can be concluded that tebuconazole increases female AGD. However, the dose at which this occurs is debatable . It would likely be beneficial to test this in a large developmental toxicity study as the OECD EOGRTS. This study finds a dose-dependent increase in nipple retention (+1.6 nipples) in male PD13 offspring with the effect reaching statistical significance at 50 mg/kg bw/d. In a previous study by the same group (Taxvig et al., 2007) also found a similar increase in NR (~ +1-1.5) at 50 and 100 mg/kg bw/d. It is noted that the levels in the controls are quite different between the two studies (0 vs 2.08). However, this is not unrealistic for an endpoint as nipple retention, not only does the levels fluctuate in controls but it is also a evaluator-dependent endpoint and therefore there should not be put too much weight on differences between studies. It is always the concurrent control, the baseline, that should be used for comparisons for this endpoint. Furthermore, clear dose response-patterns cannot allways be expected and as such it seems reasonable that Taxvig et al., 2007 found a plateaued dose-response pattern. DK-RMS finds that the effects on NR are treatment-related and it should be **emphasised** that the only 2 studies examining this endpoint found the same effects at different doses.

	Nipple retention (androgen dependent regress of the nipple anlagen) is an endpoint very different from mammary gland development with distinct MoA for the endpoints and as such there are no connection between the two and absence on effect on one cannot be used as leverage to disregard the other. Furthermore, mammary gland development has not been investigated for tebuconazole, so the effects on this endpoint are unknown. What is known for tebuconazole is that it consistently increases NR in male PND 13 offspring at 50 mg/kg bw/d. The DK-RMS proposes that based on this study no NOAEL can be set, as female AGD was significantly increased at 12.5 mg/kg bw/d. Clear endocrine-mediated effects on male NR were found at the LOAEL in male offspring at 50 mg/kg bw/d.

5)

Previous evaluation	None – publication submitted for the purpose of renewal

Study ID	B.6.6.3/05
Author(s)	Jacobsen *et al.* (2013)
Study title and journal	Persistent developmental toxicity in rat offspring after low dose exposure to a mixture of endocrine disrupting pesticides. Reproductive Toxicology, 34(2), 237-250.
Martrix ID	50
Test substance	Tebuconazole (and other pesticides)
Purity (%) **Batch no.**	98.5 Not specified
GLP	No
Guideline	No
Deviation	n/a
UK-RMS Reliability	Reliable with restrictions – low number of exposed dams; high variability; reporting shortcomings; lack of maternal toxicity (reported in other studies).
DK-RMS reliability	Reliable with restrictions – 6-8 litters. Notes: DK-RMS does not find the lack of maternal toxicity at 50 mg/kg unexpected, it is consistent with several other studies.
Relevance to hazard assessment	Relevant with restrictions

Methods

In a similar investigation from the same laboratory, pregnant Wistar female rats (10-12/dose group) were treated by oral gavage with 12.5 or 50 mg/kg bw/d tebuconazole from GD 7 to PND 16. Offspring (6-8 viable litters) were investigated at different time points for potential effects on puberty onset, reproductive organs (including sperm motility), thyroid, developmental neurotoxicity (behavioural, motor activity and learning & memory testing), mating behaviour and sex hormones.

UK RMS Results and conclusions

There were no effects on dam body weight gain, litter size or pup mortality. The UK RMS notes that the lack of maternal toxicity at the top dose of 50 mg/kg bw/d is inconsistent with the findings of several regulatory developmental toxicity studies.

In offspring, there were no effects on organ weights and histology, semen quality, sex hormone levels, onset of puberty (data not shown), mating behaviour or behaviour/learning up to the top dose of 50 mg/kg bw/d. The only exceptions were the increased liver weight in PND 16 male offspring at the high dose level (but no effect in adult offspring and no accompanying histological findings) and the increased total motor activity in adult female offspring and the increased swim length and latency in male offspring at the low dose. In the absence of histopathology, the increased liver weight is not considered adverse. The effects on motor activity and swim length noted only at the low dose are not considered to be treatment-related.

Table 6.6-79. Absolute male organ weights on PD 16 and in adult male rats (PD 260–280) exposed to the pesticides singly or in mixture during foetal and neonatal life.

Male offspring PD 16										
1: Control	15	30.8 ± 5.7	103 ± 15	23.3 ± 2.3	10.6 ± 2.5	10.4 ± 3.6	26.5 ± 5.8	1.7 ± 0.4	786 ± 128	4.4 ± 0.7
2: Pestimix-14.6	16	28.9 ± 2.8	101 ± 11	**20.3 ± 2.5*,##**	8.8 ± 1.7	8.0 ± 2.0	22.7 ± 3.8	1.5 ± 0.4	735 ± 68	4.0 ± 0.6
3: Pestimix-29.2	9	30.2 ± 3.9	**109 ± 13#**	**20.8 ± 2.2#**	**8.5 ± 1.9#**	8.6 ± 1.8	26.4 ± 6.7	1.5 ± 0.4	811 ± 120	4.4 ± 0.8
4: Pestimix-43.8	12	30.7 ± 4.5	**110 ± 15#**	**19.4 ± 1.7***,###**	**7.1 ± 1.9***,###**	**7.2 ± 1.8**,##**	24.3 ± 5.6	1.5 ± 0.6	811 ± 123	4.4 ± 1.6
11: Tebu-12.5	8	28.7 ± 3.8	104 ± 16	22.4 ± 2.4	12.0 ± 2.6	9.8 ± 3.2	25.0 ± 3.6	1.7 ± 0.4	746 ± 96	4.1 ± 1.2
12: Tebu-50	5	30.5 ± 2.8	105 ± 8	22.0 ± 2.7	9.8 ± 2.9	11.5 ± 2.2	24.5 ± 2.2	1.7 ± 0.3	**839 ± 99##**	5.3 ± 1.2
Adult male offspring										
1: Control	16	497 ± 34	3.89 ± 0.31	0.70 ± 0.07	0.63 ± 0.15	1.91 ± 0.37	1.38 ± 0.19	0.22 ± 0.07	13.2 ± 1.4	22 ± 3
2: Pestimix-14.6	18	**458 ± 29#**	3.88 ± 0.24	0.69 ± 0.07	0.67 ± 0.17	1.94 ± 0.30	1.27 ± 0.17	0.24 ± 0.08	11.6 ± 0.9	24 ± 10
3: Pestimix-29.2	12	469 ± 50	4.03 ± 0.69	0.74 ± 0.11	0.63 ± 0.12	2.11 ± 0.40	1.28 ± 0.19	0.18 ± 0.07	12.0 ± 1.8	24 ± 4
4: Pestimix-43.8	16	457 ± 32	4.01 ± 0.26	0.69 ± 0.06	**0.47 ± 0.19#**	2.08 ± 0.38	**1.15 ± 0.23*,#**	0.21 ± 0.06	12.0 ± 1.2	20 ± 3
11: Tebu-12.5	8	**455 ± 27#**	3.84 ± 0.41	0.69 ± 0.12	0.59 ± 0.13	1.80 ± 0.29	1.24 ± 0.16	0.17 ± 0.06	11.3 ± 0.8	21 ± 2
12: Tebu-50	8	481 ± 62	3.83 ± 0.22	0.66 ± 0.03	0.55 ± 0.11	1.92 ± 0.41	1.23 ± 0.19	0.16 ± 0.02	12.7 ± 1.9	24 ± 4

Bulbo: glandula bulbocavernosus; LABC: levator ani/bulbocavernosus muscle. Statistically significant differences between controls and exposed are marked with asterisks which indicate significance levels: *indicates $p < 0.05$; ** indicates $p < 0.01$; *** indicates $p < 0.001$. p values result from ANCOVA using body weights as a covariate followed by Dunnett's test on all 14 groups. # Indicates significantly different from controls in a model including only control and different doses of the same compound or the mixture using Dunnett's post hoc test. # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$. All significant results are written in bold.

Table 6.6-80. Total motor activity levels in adult male and female rat offspring exposed to the pesticides singly or in mixture during foetal and neonatal life.

	Male		**Female**	
	No. animals (litters)	**Activity count +std.dev.**	**No. animals (litters)**	**Activity count +std.dev.**
1: Control	16(14)	977+138	18(15)	1339+79
2: Pestimix-14.6	18(17)	1285+97	18(17)	1591+110
3: Pestimix-29.2	12(9)	1137+175	10(8)	1145+82
4: Pestimix-43.8	16(14)	1142+128	12(11)	1463+65
11: Tebuconazole-12.5	10(8)	1121+113	8(7)	**1938+135****
12: Tebuconazole-50	8(6)	980+104	8(6)	1524+221

Data is shown as group means + standard deviation, and for each dose group the tested number of animals and the number of litters they represent is shown. Statistically significant differences between controls and exposed groups are marked in bold. Asterisks indicate significance levels: * p<0.05, ** p<0.01. An overall significant difference between males and females was seen in the data (p<0.0001). Statistically significant differences between males and females from the same dose group were seen in groups 1, 2, 4, 7, 8, 9, 11, 12 and 14 but are not marked in the table.

Table 6.6-81. Swim lengths and latencies in male and female rat offspring exposed to the pesticide singly or in mixture during foetal and neonatal life.

MALE		Swim length								Swim latency							
	n	day 1	day 2	day 3	day 4	day 5	day 6	day 7	total	day 1	day 2	day 3	day 4	day 5	day 6	day 7	total
1: Control	10 (10)	1416+ 319	1411+ 458	880+ 459	650+ 468	562+ 329	312+ 168	273+ 117	5504+ 1760	50+ 10.4	45+ 13.4	34+ 16.3	26+ 16.3	23+ 10.5	15+ 7.0	14+ 8.3	206+ 57
2: Pestimix-14.6	10 (10)	1642+ 184	1358+ 445	867+4 85	514+2 63	518+2 22	356+ 175	299+ 129	5553+ 1096	55+ 7.2	44+ 14.8	32+ 15.9	22+ 9.9	23+ 9.6	18+ 7.8	15+ 5.8	210+ 34
3: Pestimix-29.2	10 (9)	1481+ 261	1608+ 367	1154+ 496	829+4 02	631+3 65	514+ 321	448+ 263	6665+ 1662	50+ 9.5	50+1 0.6	38+ 15.8	31+ 12.0	25+ 11.6	22+ 10.3	19+ 8.2	235+ 50
4: Pestimix-43.8	10 (10)	1571+ 312	1544+ 384	1056+ 571	911+4 34	785+4 07	580+ 436	627 +604	**7074+ 2052***	54+ 7.9	50+ 10.5	37+ 17.4	36+ 15.4	31+ 15.2	25+ 14.0	**26+ 18.3***	**259+ 63***
11: Tebu-12.5	5(5)	1778+ 328	1708+ 356	1500+ 477	1074+ 620	1024+ 836	916+ 698	**756+ 403***	**8756+ 2924****	56+ 5.3	51+ 8.8	50+ 11.3	37+ 16.8	37+ 22.6	38+ 21.4	**31+ 14****	**300+ 78****
12: Tebu-50	5(5)	1515+ 129	1536+ 615	882+6 29	642+4 35	718+6 83	376+ 311	293+ 106	5962+ 1868	50+ 3.2	50+ 16.7	33+ 18.4	27+ 16.0	28+ 23.7	18+ 11.8	14+ 5.1	220+ 54

FEMALE		Swim length								Swim latency							
	n	day 1	day 2	day 3	day 4	day 5	day 6	day 7	total	day 1	day 2	day 3	day 4	day 5	day 6	day 7	total
1: Control	10 (10)	1593+ 192	1222+ 357	911+ 346	586+ 235	669+ 398	617+ 305	574+ 277	6175+ 473	56+ 5.7	45+ 11.9	35+ 12.9	26+ 9.5	30+ 15.7	28+ 12.8	26+ 13.9	249+ 58
2: Pestimix-14.6	10 (10)	1469+ 373	1215+ 271	891+3 39	640+2 82	553+3 81	674+ 416	741+ 355	6184+ 497	52+ 11.3	45+ 11.6	35+ 11.5	28+ 10.0	25+ 14.6	28+ 15.7	30+ 11.8	247+ 62
3: Pestimix-29.2	10 (8)	1540+ 233	1413+ 382	1156+ 331	918+ 417	877+ 408	894+ 419	775+ 425	7578+ 496	53+ 6.7	51+ 11.2	50+ 11.5	41+ 16.9	43+ 18.0	40+ 16.1	34+ 15.9	314+ 58
4: Pestimix-43.8	10 (10)	1484+ 257	1246+ 397	1017+ 582	890+ 352	753+ 436	698+ 502	505+ 299	6597+ 738	51+ 7.3	44+ 12.1	36+ 16.5	35+ 12.9	29+ 15.6	29+ 17.6	23+ 14.0	250+ 73
11: Tebu-12.5	5(4)	1786+ 194	1511+ 141	1314+ 86	905+ 242	810+ 235	821+ 232	1043+2 81	8191+ 790	59+ 0.9	54+ 5.9	50+ 8.8	36+ 9.6	33+ 7.9	35+ 8.7	41+ 9.1	310+ 21
12: Tebu-50	5(5)	1429+ 273	1101+ 469	1065+ 312	876+ 466	650+ 560	916+ 581	846+ 546	6885+ 2832	52+ 11.0	40+ 15.2	44+ 13.4	33+ 12.6	29+ 19.5	36+ 17.0	33+ 17.5	270+ 87

Overall, perinatal exposure to tebuconazole up to 50 mg/kg bw/d did not cause effects on puberty onset, reproductive organs (including sperm motility), thyroid, developmental neurotoxicity (behavioural, motor activity and learning & memory testing), mating behaviour and sex hormones in offspring. On this basis, a NOAEL for developmental toxicity of 50 mg/kg bw/d is identified from this study in rats. Such NOAEL is consistent with the overall developmental NOAEL of 30 mg/kg bw/d for the rat and 10 mg/kg bw/d for the rabbit and mouse identified from the regulatory studies.

(Jacobsen *et al.,* 2013)

B.6.6.3/05 Discussion and conclusion by DK-RMS:	DK-RMS notes that a lack of effect on maternal bw at 50 mg/kg bw/d is very possible. Guideline studies have not investigated a dose level of 50 mg/kg bw/d. There were some effects at 60 mg/kg bw/d on bw in B.6.6.2.1.1/02 and more at 120 mg/kg bw/d but that does not discredit that there were no effects on bw at 50 mg/kg bw/d In their conclusion the UK-RMS does not include the effects on male offspring swim length and latency in the low dose group. These effects may be chance findings but it is also possible that the endocrine activity of tebuconazole indeed altered male brain development in the direction of the female brain. Such subtle and sometimes non-monotonic effects have been reported for other conazoles and endocrine disruptors in general and for tebuconazole very few studies have examined such potential effects, but some signs indication of some developmental neurotoxicity effects were also evident in a regulatory DNT study (B.6.6.2.1.2/01) as well as in a DNT study by B.6.6.2.1.2/01.

6)

Previous evaluation	None – publication submitted for the purpose of renewal

Study ID	B.6.6.3/06
Author(s)	Overgaard *et al.* (2013)
Study title and journal	The effect of perinatal exposure to ethinyl oestradiol or a mixture of endocrine disrupting pesticides on kisspeptin neurons in the rat hypothalamus. NeuroToxicology, 37, 154-162.
Matrix ID	51
Test substance	Tebuconazole (and other pesticides)

Purity (%) **Batch no.**	98.5% (stated in Hass et al 2012 and Jacobsen et al 2013 publications from same study) Not specified
GLP	No
Guideline	No
Deviation	n/a
Reliability	Reliable with restrictions – low number of exposed dams; high variability; reporting shortcomings.
Relevance to hazard assessment	Relevant with restrictions

Methods
Another investigation from the same laboratory, reported more results from the same study as described by Jacobsen et al 2013. Pregnant Wistar female rats (10-12/dose group) were treated by oral gavage with 12.5 or 50 mg/kg bw/d tebuconazole from GD 7 to PND 16, and the present publication showed results for potential effects on puberty onset (balano-preputial separation in males and vaginal opening in females), and levels of kisspeptin (a positive regulator of the hypothalamic–pituitary–gonadal axis, which plays a key role in the initiation of puberty) mRNA in the hypothalamus (PND50).

UK-RMS Results and conclusions
Perinatal exposure to tebuconazole up to 50 mg/kg bw/d had no effects on all of the parameters investigated. Nominal delays of 2 days on vaginal opening in female offspring of both exposure groups were seen, there was no effects on bw on PD 28 or in adulthood. On this basis, a NOAEL for developmental toxicity of 50 mg/kg bw/d is identified from this study in rats. Such NOAEL is consistent with the overall developmental NOAEL of 30 mg/kg bw/d for the rat and 10 mg/kg bw/d for the rabbit and mouse identified from the regulatory studies.
(Overgaard *et al.,* 2013)

B.6.6.3/06 Discussion and conclusion by DK-RMS:	The only endpoints reported for this study were PPS (data not shown), VO and mRNA in the hypothalamus. It is thus limited how many endpoints a NOAEL derived from this study covers. DK-RMS notes that there were nominal delayes in female offspring VO in the absence of effects on bw. Although non significant (the study was not adequately powered for such endpoint), delays in puberty are consistent with effects observed in both pubertal and developmental neurotoxicity studies of tebuconazole. However, in the present study this effect was not in the presence of an effect on bw, providing additional support that this is a specific effect of tebuconazole on the time of sexual maturation rather than a non-specific effects caused by generally delayed postnatal development.

7)

Previous evaluation	None – publication submitted for the purposes of renewal

Study ID	B.6.6.3/07
Author(s)	Taxvig *et al.* (2007)
Study title and journal	Endocrine-disrupting activities *in vivo* of the fungicides Tebuconazole and Epoxiconazole. Toxicological Sciences, 100(2), 464-473.
Matrix ID	54
Test substance	Tebuconazole (and epoxiconazole)
Purity (%) **Batch no.**	98 Not specified
GLP	No
Guideline	No
Deviation	n/a
UK Reliability	Reliable with restrictions – low number of dams subject to caesarean section; high variability; reporting shortcomings; inconsistent findings with subsequent study by the

	same authors.
RMS-DK reliability	Reliable with restrictions – group size 6-8 for caesarean section. Notes: RMS-DK does not find the study to have any higher variability than guideline studies investigating similar endpoints that are variable such as hormone levels. That is simply the nature of some endpoints; hormone levels exhibits high variation between individuals as well as over time. RMS-DK finds the results to be comparable to what was found in Taxvig et al., 2008 and Hass et al., 2012, similar studies by the same group (see discussion by DK-RMS of Hass et al., 2012).
Relevance to hazard assessment	Relevant with restrictions

Methods
In another investigation from the same laboratory, pregnant Wistar female rats (20/dose group) were treated by oral gavage with 50 or 100 mg/kg bw/d tebuconazole from GD 7 to PND 16. On GD 21, 8-12 dams were randomly selected for caesarean section.

Table 6.6-82. Dose and group size.

Dose [mg/kg bw/day]	Number of treated rats	Number of dams with viable litters
0	24	19
50	20	19
100	20	18

Table 6.6-83. Treatment period: GD 7 to post natal day 16

Dose [mg/kg bw/day]	Number of dams for caesarean section [1]	Pregnant dams
0	8 or 12	6
50	8 or 12	7
100	8 or 12	8

GD: gestation day
[1] On GD 21, 8 or 12 dams in each dose were randomly selected for section. Additional sections on GD 24–25 had to be performed on two dams in the Teb-100 group, because the dams were unable to give birth and were diagnosed to have dystocia.

The other dams were allowed to deliver and rear their pups. Offspring were investigated for potential effects on AGD (GD21 and PND1), nipple retention (PND13), external genitals (PND16), reproductive organs (PND16), thyroid and adrenals (PND16), semen quality (7 month-old), sex steroid and T3 hormone levels (GD21 and PND16).

UK-RMS Results and conclusions
The high dose of tebuconazole decreased maternal weight gain during pregnancy, probably due to effects on both the dam and the uterine content. Furthermore, the high dose of tebuconazole increased gestational length, caused loss of foetuses, and postnatal death of the pups. Many of the dead foetuses (27 of 128) had died very late in the gestation period. Tebuconazole decreased male and female foetal weight on GD 21 at the high dose.

Exposure to tebuconazole increased AGD in female foetuses at GD 21 at 50 and 100 mg/kg bw/d and in new-born (PND1) female offspring at the top dose (100 mg/kg bw/d). The UK RMS notes the inconsistent results on AGD at GD21 and PND1 at 50 mg/kg bw/d. The UK RMS also notes that in a subsequent study by the same authors (Taxvig *et al.,* 2008; see below), there was no effect on AGD on GD21 at 50 mg/kg bw/d. The UK RMS also notes that there were no effects on onset of puberty and mating behaviour up to 50 mg/kg bw/d in subsequent studies performed by the same research group (Jacobsen *et al.,* 2013; Overgaard *et al.,* 2013). Therefore, the reported findings on AGD are either not treatment-related or of no toxicological significance.

A statistically significant effect on nipple retention on PND 13 was seen in the male pups exposed to tebuconazole at both dose levels (3.43 and 3.07 areolae at 50 and 100 mg/kg bw/d, respectively vs 2.08 areolae

in controls), but no clear dose-response was apparent. Given the lack of dose-response the UK RMS concludes that the reported effect on nipple retention is not treatment-related.

Table 6.6-84. Pregnancy and litter data. Data represent group means based on litter means ± SD

	Control	Teb-50	Teb-100
Dams and litters			
No. of dams (viable litters)	$N = 13$ (13)	$N = 12$ (12)	$N = 10$ (8)[a]
Maternal weight gain GD 7–21	85.38 ± 11.9	77.17 ± 14.4	**61.00 ± 12.5***
Maternal weight gain GD 7–PND 1	20.62 ± 7.2	17.58 ± 6.8	**13.50 ± 10.6***
Body weight gain PND 1–13	7.53 ± 16.3	7.75 ± 16.8	−4.57 ± 12.9
Gestation length (days)	22.46 ± 0.5	22.67 ± 0.5	**23.40 ± 1.2****
% Postimplantation loss	6.55 ± 5.1	10.31 ± 11.2	**27.32 ± 23.5***
% Perinatal loss	9.67 ± 8.0	13.37 ± 12.5	**54.97 ± 36.9****
Litter size	11.15 ± 1.7	10.75 ± 3.6	8.75 ± 3.8
Born alive per litter	10.92 ± 1.7	10.67 ± 3.7	8.38 ± 3.8
Born dead per litter	0.23 ± 0.4	0.08 ± 0.3	0.37 ± 0.7
% Postnatal death	3.39 ± 5.6	3.36 ± 7.04	**27.00 ± 37.5***
% Males	44.76 ± 17.6	56.42 ± 11.7	40.36 ± 18.6
Offspring (data from viable litters)			
Birth weight (g)	5.53 ± 0.3	5.64 ± 0.5	5.63 ± 0.8
Body weight PND 13 (g)	23.25 ± 2.6	21.59 ± 4.1	22.39 ± 5.02
Male AGD (mm)	3.41 ± 0.2	3.39 ± 0.1	3.51 ± 0.2
Male AGD per cubic root body weight	1.92 ± 0.1	1.90 ± 0.1	1.96 ± 0.1
Female AGD (mm)	1.72 ± 0.1	1.80 ± 0.1	**1.91 ± 0.1***
Female AGD per cubic root body weight	0.98 ± 0.03	1.02 ± 0.1	**1.09 ± 0.1***
No. areolas males	2.08 ± 0.6	**3.43 ± 0.9****	**3.07 ± 2.5****
No. areolas females	12.5 ± 0.4[c]	12.46 ± 0.4	12.31 ± 0.4
GD 21 cesarean section			
No. of dams	$N = 6$	$N = 7$	$N = 8 + 2$[a]
Maternal body weight (g)	307.17 ± 22.4	297.00 ± 27.2	**281.00 ± 26.5***
Adjusted body weight (g)	232.70 ± 14.9	234.00 ± 16.8	223.31 ± 20.4
No. of implantations	12.50 ± 2.1	12.00 ± 3.2	11.60 ± 1.5
No. of fetuses	11.67 ± 2.1	11.14 ± 3.6	9.40 ± 2.1
% Postimplantation loss	6.45 ± 7.9	9.10 ± 11.3	**21.54 ± 9.4***
% Late resorptions	1.28 ± 3.1	2.38 ± 6.3	6.14 ± 4.2
% Very late resorptions	0.0 ± 0.0	2.38 ± 6.3	2.39 ± 4.2
% Males	56.01 ± 17.2	46.22 ± 20.9	49.74 ± 20.6
Fetal weight male (g)	4.45 ± 0.3	3.84 ± 0.7	**3.44 ± 0.9****
Fetal weight female (g)	4.18 ± 0.4	3.61 ± 0.6	**3.40 ± 0.9***
No. of litters for AGD[b]	$N = 3$	$N = 4$	$N = 4$
Male AGD (mm)	3.39 ± 0.3	3.50 ± 0.0	3.29 ± 0.4
Male AGD per cubic root body weight	2.08 ± 0.1	2.25 ± 0.2	**2.30 ± 0.1***
Female AGD (mm)	1.65 ± 0.1	**1.87 ± 0.2***	**2.02 ± 0.1****
Female AGD per cubic root body weight	1.04 ± 0.1	1.23 ± 0.2	**1.43 ± 0.2****

Values shown in bold are statistically significantly different compared to control, * $p < 0.0.5$ and ** $p < 0.01$.

[a] Because of problems with parturition caesarean section (CS; GD 23–25) was performed on two additional dams in the Teb-100 group.

These data were included in the analysis of GD 21 CS data.

[b] AGDs were only measured in the second set of animals.

A statistically significant increased liver weight is observed at 100 mg/kg tebuconazole. No effects on the reproductive organ weights or body weight were observed for either dose of tebuconazole. Weights of female reproductive organs were unaffected.

Table 6.6-85. Effects of Tebuconazole on Male and Female Organ Weights PND 16

Male	Control	Teb-50 mg	Teb-100 mg
Body weights (g)	28.9 ± 0.4 (42)	26.6 ± 0.7 (46)	27.9 ± 1.1 (25)
Right testis (mg)	54.7 ± 0.7 (43)	53.0 ± 1.7 (45)	56.6 ± 2.8 (25)
Left testis (mg)	54.4 ± 0.9 (43)	52.7 ± 1.7 (44)	54.5 ± 2.9 (25)
Epididymides (mg)	20.8 ± 0.5 (24)	20.1 ± 0.7 (24)	21.4 ± 1.1 (14)
Ventral prostate (mg)	12.4 ± 0.4 (25)	12.5 ± 0.8 (22)	13.2 ± 1.1 (14)
Seminal vesicles (mg)	8.7 ± 0.5 (24)	8.3 ± 0.5 (24)	9.4 ± 0.9 (13)
LABC (mg)	26.0 ± 0.1 (12)	22.3 ± 1.4 (11)	25.3 ± 1.8 (7)
Bulbourethral gl (mg)	1.6 ± 0.1 (11)	1.5 ± 0.1 (12)	1.7 ± 0.2 (7)
Thyroid (mg)	3.6 ± 0.2 (31)	3.8 ± 0.2 (33)	3.6 ± 0.2 (18)
Adrenals (mg)	8.5 ± 0.3 (26)	7.9 ± 0.5 (24)	8.7 ± 0.9 (14)
Kidneys (mg)	295.5 ± 7.9 (13)	272.2 ± 18.4 (12)	295.3 ± 27.5 (7)
Liver (mg)	735.6 ± 17.8 (25)	702.3 ± 40.6 (24)	795.2 ± 49.9* (14)
Female			
Body weights (g)	29.0 ± 0.7 (24)	26.5 ± 1.2 (21)	28.2 ± 1.9 (12)
Thyroid (mg)	3.9 ± 0.6 (12)	4.4 ± 0.3 (11)	3.9 ± 0.5 (6)
Uterus (mg)	18.7 ± 0.6 (13)	19.1 ± 1.3 (12)	19.7 ± 1.5 (6)
Ovary (mg)	5.5 ± 0.3 (9)	5.1 ± 0.3 (7)	5.7 ± 0.4 (5)

Note. LABC, levator ani/bulbocavernosus muscles. Data represent least squares means ± SEM; total number n in parenthesis; Teb-50 and Teb-100 = tebuconazole 50 and 100 mg/kg bw/d.
*Statistically, significantly different compared to controls ($p < 0.05$).

There were no effects on semen quality. The levels of testicular testosterone, progesterone, and 17α-hydroxyprogesterone were affected in male foetuses taken by caesarean section on GD 21 at both dose levels. In plasma from tebuconazole-dosed dams at GD 21, a marked increase in the progesterone level as well as a significant decrease in T3, were seen at the top dose. In absence of effects on thyroid hormone levels in other similar investigations by the same research group and given the lack of effects on thyroid weight or histopathology in this study and other studies by the same laboratory, the decrease in T3 at the top dose of 100 mg/kg bw/d is considered to be a chance finding.

Table 6.6-86. Testicular Hormone Levels in Male Foetuses at GD 21.

	17α-hydroxyprogesterone (pg/testis)	Testosterone (ng/testis)	Progesterone (ng/testis)	Testosterone production (ng/testis)	Progesterone production (ng/testis)
Control	1.95 ± 0.54 (4)	1.75 ± 0.71 (5)	0.037 ± 0.025 (5)	3.95 ± 1.71 (6)	0.02 ± 0.01 (6)
Tebuconazole 50 mg/kg	**8.39 ± 2.59*** (7)	1.25 ± 0.40 (7)	**0.103 ± 0.035*** (7)	4.77 ± 3.49 (6)	0.29 ± 0.62 (6)
Tebuconazole 100 mg/kg	**6.59 ± 3.88*** (9)	**0.88 ± 0.46*** (9)	0.084 ± 0.063 (9)	3.34 ± 2.59 (5)	0.04 ± 0.03 (5)

Note. Testes from male foetuses (GD 21) exposed to tebuconazole (50 or 100 mg/kg) were extracted with diethyl ether, and hormone levels were analyzed as described in ''Materials and Methods'' section. Data represent the mean ± SD; total number n in parenthesis; Values shown in bold are statistically significantly different compared to control, * $p < 0.0.5$.

Table 6.6-87. Plasma Hormone Levels in Dams at GD 21.

	T_3 mean (nM)	T_4 mean (nM)	Testosterone mean (nM)	Progesterone mean (nM)
Control	2.38 ± 0.48 (7)	53.53 ± 17.45 (7)	0.40 ± 0.25 (6)	48 ± 32 (6)
Tebuconazole 50 mg/kg	2.36 ± 0.24 (7)	53.14 ± 11.95 (7)	0.22 ± 0.09 (7)	113 ± 114 (7)
Tebuconazole 100 mg/kg	**1.99 ± 0.23*** (8)	38.26 ± 10.74 (8)	0.32 ± 0.17 (8)	**354 ± 163*** (8)

Note. Data represent the mean ± SD; total number n in parenthesis; Values shown in bold are statistically significantly different compared to control, * $p < 0.0.5$.

Table 6.6-88. Hormone Levels in Pups PND 16.

	Estradiol	Testosterone
	Mean (pg/ovary)	Mean (ng/ml) (male plasma)
Control	8.40 ± 3.90 (13)	0.14 ± 0.18 (12)
Tebuconazole 50 mg/kg	8.40 ± 7.00 (12)	0.17 ± 0.18 (12)
Tebuconazole 100 mg/kg	5.60 ± 4.60 (7)	0.11 ± 0.09 (7)

Overall, perinatal exposure to tebuconazole caused maternal toxicity and pup mortality at the top dose of 100 mg/kg bw/d and effects on a number of sex steroid hormones in pups and dams from 50 mg/kg bw/d. Therefore, a marginal LOAEL of 50 mg/kg bw/d can be identified for developmental toxicity from this study in rats based on effects on sex steroid hormones. Such LOAEL is not inconsistent with the overall developmental NOAEL of 30 mg/kg bw/d for the rat and 10 mg/kg bw/d for the rabbit and mouse identified from the regulatory studies.

(Taxvig *et al.,* 2007)

B.6.6.3/07 Discussion and conclusion by DK-RMS:	**Materal effects at the LOAEL** Maternal : post-implantation loss and postnatal death, prolonged gestation and late gestation resorptions, reduced maternal weight gain (29-34% at high dose). Moreover, two dams in the 100 mg/kg group were unable to give birth due to dystocia. Reduced dam weight (8% reduction at high dose GD 21), no change in dam weight adjusted for litter and uterine weight. Tebuconazole caused marked reproductional toxicity at 100 mg/kg bw/d with high postimplanation loss and postnatal deaths as well as prolonged gestation and late gestation resorptions. These effects are consistent with adverse effects on pregnancy due to alterations of steroid hormones which was also shown in this study. In particular, the seven-fold increase in late-gestation progesterone levels is causative of the dystocia. In addition the offspring showed signs of endocrine disruption with alterations in testicular testosterone levels (50 and 100 mg/kg bw/d) and progesterone levels (100 mg/kg bw/d). The changed uterine hormonal environment gave rise to altered endocrine tissue development in the offspring with virilised females with longer AGD and feminized males with increased nipple retention on PD13 (RMS-DK do not consider the plateau effect of 3.42 nipples at 50 mg/kg bw/d and 3.07 at 100 mg/kg bw/d to be of significant importance to disregard the findings). DK-RMS also notes that there has been some offspring loss at 100 mg/kg bw/d this dose). A LOAEL of 50 mg/kg bw/d can be identified from this study based on effects on sex steroid hormone and increase nipple retention in male offspring. See Hass et al., 2012 for discussions on effects on AGD and NR (nipple retention) in the series of studies by this research group. RMS-DK is of the opinion that the discrepancies between the effects at different doses is not of major importance. The two studies investigating NR finds increased NR in male PD 13 offspring at 50 mg/kg bw/d. See Hass et al., 2012 for DK-RMS discussion of why effects on AGD does not necessarily give rise to effects also on mating behaviour and puberty. In and of itself, altered AGD is indicative of endocrine disruption. In addition, in this study there were nominal delays in VO at the highest dose of 50 mg/kg bw/d further supporting endocrine disruption in this study.

8)

Previous evaluation	None – publication submitted for the purposes of renewal

Study ID	B.6.6.3/08
Author(s)	Taxvig *et al.* (2008)
Study title and journal	Endocrine-disrupting properties *in vivo* of widely used azole fungicides. International Journal of Andrology, 32(2), 170-177.
Matrix ID	53
Test substance	Tebuconazole (and other azole fungicides)
Purity (%) **Batch no.**	98 Not specified
GLP	No
Guideline	No
Deviation	n/a
Reliability	Reliable with restrictions – low number of exposed dams; high variability; reporting shortcomings.
Relevance to hazard assessment	Relevant with restrictions

Methods

In another investigation from the same laboratory, tebuconazole was examined in the Hershberger assay in castrated male rats at 50, 100 and 150 mg/kg bw/d given orally by gavage. Following treatment, the weights of reproductive organs, serum hormone levels (LH, FSH and T4) and prostate gene expression of androgen-regulated genes were evaluated.

Table 6.6-89. Dose and group size.

Group name (n = 6 / group)		Tebuconazole (mg/kg bw/day)	Testosterone (mg/kg/day s.c.)	Flutamide (mg/kg bw/day orally)
Intact animal	Intact	0	0	0
Castrated control	Castrated	0	0	0
Positive control	Castrated	0	0.5	10
Control	Castrated	0	0.5	0
Tebu 50	Castrated	50	0.5	0
Tebu 100	Castrated	100	0.5	0
Tebu 150	Castrated	150	0.5	0

In addition, in a developmental toxicity study, pregnant female Wistar rats (9) were dosed by oral gavage with tebuconazole at 50 mg/kg bw/d from gestational day (GD) 7 to GD 21. Caesarean sections were performed on dams at GD 21. GD 21 foetuses were investigated for potential effects on AGD and sex steroid hormone levels.

Table 6.6-90. Dose and group size.

Tebuconazole (mg/kg bw/day)	Number of treated rats	Number of time-mated pregnant rats
0	10	6
50	10	9

Results and conclusions

Hershberger assay

The weights of the reproductive organs (prostate, seminal vesicle, LABC and bulbourethralis glands) and hormone levels (LH, FSH, T4) were unaffected by tebuconazole. The expression in the prostate of one gene (OCD) was decreased at the highest dose. Overall, tebuconazole was negative in this Hershberger assay.

Table 6.6-91. Hersheberger assay - Body weight, organ weights and serum hormone levels

	Intact control	Castrated control	Castrated rats given testosterone propionate				
			Control	Flutamide	Tebu 50	Tebu 100	Tebu 150
Body weight (g)	183 ± 14	166 ± 3	165 ± 10	165 ± 18	164 ± 16	165 ± 14	170 ± 15
Prostate (mg)	87.5 ± 10.8*	5.7 ± 3.6*	49.2 ± 18.0	11.1 ± 5.9*[a]	57.2 ± 13.6	51.4 ± 10.8	61.0 ± 8.8[a]
Seminal vesic. (mg)	114.0 ± 36.7	12.5 ± 3.0*	95.9 ± 39.7	20.1 ± 3.9*	106.2 ± 12.4	90.5 ± 7.7	101.4 ± 11.1
Musc. lev. ani (mg)	229 ± 24	93 ± 11*	199 ± 12	124 ± 24*	202 ± 38	216.7 ± 22.6	222 ± 27
Bulbourethral gl. (mg)	15.8 ± 4.1*	1.7 ± 0.7*[a]	9.0 ± 2.2	1.8 ± 0.8*	10.5 ± 1.3	10.4 ± 1.3	10.7 ± 2.0
Pituitary (mg)	6.2 ± 0.5	7.6 ± 1.0	6.4 ± 0.6	6.6 ± 1.9	6.5 ± 0.7	6.7 ± 0.9	7.5 ± 1.0
Thyroid (mg)	9.5 ± 3.8	9.1 ± 5.0	8.0 ± 2.0	8.3 ± 1.1	8.0 ± 1.0	8.2 ± 1.8	8.4 ± 3.0
Liver (g)	8.2 ± 0.6*	7.2 ± 0.3	6.9 ± 0.5	7.7 ± 0.9	7.2 ± 0.8	7.4 ± 0.7	8.5 ± 1.0*
Kidney (g)	1.5 ± 0.1[a]	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	1.4 ± 0.2
Serum hormone levels							
LH (ng/mL)	1.20 ± 0.54	2.0 ± 18.9*	0.8 ± 1.3	15.8 ± 6.2*	0.9 ± 0.8	0.5 ± 0.6	1.0 ± 1.0
FSH (ng/mL)	9.80 ± 0.97*	32.2 ± 5.6*	20.8 ± 3.2	36.9 ± 5.0*	24.2 ± 3.7	19.7 ± 5.8	21.6 ± 3.5
T4 (nm)	127 ± 41	110 ± 28	140 ± 41	123 ± 23	145 ± 33	141 ± 39	146 ± 42

Data represents mean ± SEM (n = 6). [a]n = 5; one-way anova. *Statistical significance compared to controls by Dunnett's test ($p < 0.05$).

Developmental toxicity study

Tebuconazole induced a high frequency of post-implantation loss at the only tested dose of 50 mg/kg bw/d. Tebuconazole did not affect AGD on GD21 at the only tested dose of 50 mg/kg bw/day. In dams, tebuconazole decreased plasma estradiol. In male foetuses, tebuconazole increased testicular progesterone levels. No changes were observed in foetal plasma testosterone and progesterone levels or in the oestradiol levels in ovaries.

Table 6.6-92. Developmental toxicity study - Pregnancy and litter data.

GD Caesarean section	**1. Control**	**3. Tebu-50**
No. dams	6	9
Maternal body weight	2240 ± 8.3	224.7 ± 4.5
No. implantations	11.3 ± 0.8	11.3 ± 0.9
No. live foetuses	11.3 ± 0.8	10.4 ± 1.2
% post-implantation loss	0.0 ± 0.0	**11.4 ± 6.2***
% late resorptions	0.0 ± 0.0	4.4 ± 4.4
% very late resorptions	0.0 ± 0.0	2.2 ± 2.2
% males	56.5 ± 2.6	48.3 ± 7.6
Male foetal weight[a]	3.6 ± 0.2	3.9 ± 0.3
Female foetal weight[a]	3.5 ± 0.2	3.6 ± 0.2
Male AGD (mm)[b]	3.76 ± 0.08	3.75 ± 0.05
Male AGD index	2.47 ± 0.03	2.41 ± 0.07
Female AGD (mm)[b]	2.12 ± 0.03	2.12 ± 0.05
Female AGD index	1.40 ± 0.03	1.40 ± 0.05

Tebu-50, tebuconazole 50 mg/kg/day; res, resorption i.e. regression of the foetus; AGD, anogenital distance.
Data represent group means, based on litter mean ± SEM.
[a]Foetal body weight was analysed using the live number of foetuses as a covariate.
[b]AGD was analysed both with and without the cubic root of body weight as a covariate and both analyses showed that only group 5 was significantly affected.
AGD index means the AGD divided by the cubic root of body weight.
Values shown in bold are statistically significantly different compared with control, *p < 0.05 and **p < 0.01 respectively.

Table 6.6-93. Developmental toxicity study - Plasma hormone levels in dams at GD 21 and testicular hormone levels in foetuses.

	17α-Hydroxyprogesterone (ng/mL)	**Progesterone** mean (nm)	**Testosterone** mean (nm)	**Oestradiol** mean (nm)
Control	13.11 ± 4.02 (8)	95 ± 100 (9)	0.38 ± 0.32 (9)	0.036 ± 0.024(9)
Tebuconazole 50 mg/kg	17.85 ± 9.87 (8)	161 ± 126 (10)	0.29 ± 0.19 (10)	**0.015 ± 0.006*(10)**
	Progesterone (ng/testis)	**Testosterone (ng/testis)**	**Testosterone production (ng/testis)**	**Progesterone production (ng/testis)**
Control	0.08 ± 0.04 (16)	1.64 ± 0.68 (13)	4.90 ± 3.80 (6)	0.001 ± 0.00 (6)
Tebuconazole 50 mg/kg	**0.27 ± 0.12*(23)**	1.38 ± 0.88 (23)	2.50 ± 1.60 (8)	0.004 ± 0.002 (8)

Testes from male foetuses (GD 21) exposed to tebuconazole (50 mg/kg) were extracted with diethyl ether and hormone levels were analysed as described in Materials and methods. Data represent mean ± SD. Values shown in bold are statistically significantly different compared with control, *p < 0.05. ( ) = n.

Conclusion
Overall, tebuconazole showed no anti-androgenic potential in the Hershberger assay up to and including the highest tested dose of 150 mg/kg bw/d. However, tebuconazole caused a significant increase in testicular progesterone levels in male foetuses (possible indicator of demasculinization of male foetuses) at 50 mg/kg bw/d in a developmental toxicity study. In addition tebuconazole induced a high frequency of post-implantation loss and a decrease in estradiol in dams at 50 mg/kg bw/d. Overall a LOAEL for developmental toxicity of 50 mg/kg bw/d can be identified from this study in rats based on increased post-implantation loss and effects on sex steroid hormone levels. Such LOAEL is not inconsistent with the overall developmental NOAEL of 30 mg/kg bw/d for the rat and 10 mg/kg bw/d for the rabbit and mouse identified from the regulatory studies.

(Taxvig *et al.,* 2008)

B.6.6.4 Overall summary on reproductive toxicity

The reproductive toxicity of tebuconazole has been investigated in numerous regulatory studies (a rat multi-generational study, developmental toxicity and developmental neurotoxicity studies in rats and developmental toxicity studies in rabbits and mice). There are also eight publications (three *in vitro* investigations and five *in vivo* targeted developmental toxicity studies in rats) of relevance to reproductive toxicity from the open literature. Three of these (Taxvig et al 2007, Taxvig et al 2008, Hass et al 2012) are relevant as supporting information when setting NOAEL/LOAEL values for reproductive toxicity effects in rats, and these studies have therefore by the DK-RMS been included in the overview tables on the following pages.

One multi-generational study (B.6.6.1.1/01) in the rat was described in the original DAR (2006).

In rats, two developmental toxicity studies by oral administration were described in the original DAR (2006) (B.6.6.2.1.1/01 and B.6.6.2.1.1/02). In addition, a new study on maternal toxicity in pregnant rats after oral administration was submitted for the purpose of renewal (B.6.6.2.1.1/03); this study served as an investigative study of maternal toxicity and NOAELs were not derived. Two developmental neurotoxicity studies by oral administration were described in the original DAR (2006) (B.6.6.2.1.2/01 and B.6.6.2.1.2/01. In addition, a review of these developmental neurotoxicity studies was also described in the original DAR (2006) (B.6.6.2.1.2/03). Two developmental toxicity studies by dermal administration were also described in the original DAR (2006) (B.6.6.2.1.3/01 and B.6.6.2.1.3/02). There are also five oral targeted developmental toxicity studies from the open literature.

In rabbits, four developmental toxicity studies by oral administration were described in the original DAR (2006) (B.6.6.2.2.1/01; B.6.6.2.2.1/02; B.6.6.2.2.1/03 and H B.6.6.2.2.1/04). Two different strains of rabbit were used - Himalayan CHBB:HM rabbits (B.6.6.2.2.1/01) and Chinchilla rabbits (B.6.6.2.2.1/02; B.6.6.2.2.1/03 and B.6.6.2.2.1/04. The study B.6.6.2.2.1/04 served as an investigative study of maternal toxicity and NOAELs were not derived.

In mice, three developmental toxicity studies by oral administration were described in the original DAR (2006) (B.6.6.2.3.1/01; B.6.6.2.3.1/02 and B.6.6.2.3.1/03). The study B.6.6.2.3.1/02 served as a supplementary study and

whilst NOAELs were not derived, an effect on maternal toxicity was clear at the top dose. One developmental toxicity study by dermal administration was described in the original DAR (2006) (B.6.6.2.3.2/01).

B.6.6.4 Discussion and conclusion by RMS-DK:	It is proposed by the DK-RMS to classify **Tebuconazole Repr. 1B, H360F May damage fertility.** The main adverse fertility effects 1) dystocia and prolonged gestation 2) post implantation loss, and 3) effects on the reproductive system of perinatally exposed males have been assessed and compared with the CLP criteria and the conclusions are as follows: - The observed dystocia and prolonged gestation seen in rats in the absence of marked maternal systemic toxicity, supports a classification as Repr. 1B (CLP) for this effect. - The observed post implantation loss seen in rats, rabbits and mice in absence of marked maternal systemic toxicity, supports a classification as Repr. 1B (CLP) for this effect. - The observed effects on the reproductive system of developmentally exposed males are not considered sufficient for classification for fertility on its own, however the observed effects indicates a potential and in the absence of more elaborated data investigating these end points in up to date OECD compliant studies, these have been included in a WoE approach. On this basis, classification of tebuconazole for toxicity to fertility in category 1b (Repr 1b; H360F) is proposed. It is proposed by the DK-RMS to classify Tebuconazole **Repr. 1B, H360D May damage the unborn child.** The main adverse developmental effects 1) post implantation loss and perinatal death, 2) fetal/pup growth impairment, and 3) external malformations including cleft palates have been assessed and compared with the CLP criteria by the Committee and the conclusions are as follows: - The observed post implantation loss and perinatal death seen in rats, rabbits and mice in the absence of marked maternal toxicity, support a classification as Repr. 1B (CLP) for this effect. - The observed fetal/pup growth impairment seen in rats, rabbits and mice in the absence of marked maternal toxicity, support a classification as Repr. 1B (CLP) for this effect. - The presence of external malformations including cleft palates in the mouse and rabbit foetuses in the absence of marked maternal toxicity, support classification as Repr. 1B (CLP). On this basis, the classification of tebuconazole for fertility and developmental toxicity in category 1b (Repr 1b; H360FD, May damage fertility. May damage the unborn child) is appropriate. **The lowest LOAEL for reproductive toxicity across all studies was 10 mg/kg bw/d, identified in the mouse and agreed at PRAPeR Expert Meeting 49 (2-6 June 2008).**

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Table 6.6-94. Overview of Reproductive toxicity studies.

Study	Dose range tested (mg/kg bw/d)	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d)	Effects at the LOAEL	Study reference
Multi-generational Study: Rat					
Two-generation, dietary GLP OECD test guideline no. 416 (1983) Tebuconazole, batch FL 132 (mixed batches), 95.2 %. Rat Bor: WISW (SPF Cpb)	ppm: 0, 100, 300 and 1000 Equivalent to (mg/kg bw/d): 0, 9.1 – 11.1, 27.8 – 33.9 and 94.8 – 111.4 in F and 0, 7.1 – 9.2, 21.6 – 27.1 and 72.3 – 97.2 in M.	<u>Reproductive, parental and offspring</u>: 300 ppm. Equivalent to: 21.6 – 27.1 (males) and 27.8 – 33.9 (females).	<u>Reproductive, , parental and offspring</u>: 1000 ppm. Equivalent to: 72.3 - 97.2 (males) and 94.8 - 111.4 (females).	<u>Reproductive:</u> adverse effects on pre- and postnatal offspring in the first generation (lower litter size, indicating possible postimplantation loss**; i**ncreased postnatal mortality) <u>Parental and offspring</u>: Decreased food consumption is not statistically significant (8-11% of control, no change in first generation females), slightly retarded weight gains for parents and decrease in bodyweight (less than 10% and not considered toxicologically relevant in parents). Reduced birth weight (less than 10%, some generations) & bw for pups during development (15-25% at some ages). Organ weight decrease (absolute liver & kidney weight, not relative) secondary to decreased body weights in F1B parentals.	B.6.6.1.1/01

				Possible dystocia in one dam (dam was found moribund. When sacrificed there were foetuses in both uterine horns, and the placentas in one horn were found to be very thick, beige coloured and hard).	
Developmental Toxicity: Rat					
Developmental toxicity, oral gavage GLP Comparable to OECD test guideline no. 414 (1981) Tebuconazole, batch 16007/83, 93.4 % Rat WISW	0, 10, 30, 100	Maternal: 10 Developmental: 30	Maternal: 30 Developmental: 100	Maternal: Reduced body weight gains (marginal) during treatment, no change in maternal body weight. Developmental: Increased number of external malformations, higher incidence of post-implantation losses and decreased foetal body weight.	B.6.6.2.1.1/01
Developmental toxicity, oral gavage GLP OECD test guideline no. 414 (1981) Tebuconazole, batch no. 20, 98.3 % Rat Wistar/HAN	0, 30, 60, 120	Maternal: 30 Developmental: 60	Maternal: 60 Developmental: 120	Maternal: Reduced body weight gain and feed intake and increased liver weights. It is noted that the values for body weight gain corrected for uterus weight were not significantly lower at the high dose during pregnancy. Developmental: Higher incidence of resorptions, reduced ossification, decreased foetal weight and an increased	B.6.6.2.1.1/02

				incidence of skeletal variations and anomalies	
Maternal toxicity in pregnant rats, oral gavage GLP OECD test guideline not specified – investigative study Tebuconazole, batch 278679012 98.5 / 98.6 % Rat Wistar Hsd Cpb:WU	0, 120	Not applicable – investigative study	Not applicable – investigative study	Effects on body weight (gain), food and water consumption (decreased) and on clinical signs of toxicity (piloerection, increased urination), specific toxic effects were seen in the liver (slight reduction in weight accompanied by histopathology) and adrenal gland (vacuolation of zona fasciculate and zona glomerulosa cells).	B.6.6.2.1.1/03
Developmental neurotoxicity (DNT), oral dietary GLP Not in accordance with OECD test guideline (in accordance with US-EPA, OPPTS 870.6300; US-EPA, Pesticide Assessment Guidelines) Tebuconazole, batch 603-00l 3, 96.0 – 96.9 % Rat Sprague-Dawley rats (Crl:CD®BR VAF/Plus®)	ppm: 0, 100, 300, 1000. Equivalent to (mg/kg bw/d) Gestation days 6-21: 0, 8.8, 22.0 and 65.0; Lactation days 1-12: 0, 16.3, 41.3 and 125.4.	<u>Parental and developmental:</u> 22 and 41.3 during gestation and lactation, respectively (300 ppm)	<u>Parental and developmental:</u> 65 and 125.4 during gestation and lactation, respectively (1000 ppm)	<u>Parental:</u> Reduced body weight and feed consumption, prolonged gestation, two maternal deaths/moribund sacrifices related to dystocia. <u>Developmental:</u> Mortality, decreased number of live born (-6% compared to control), decreased viability index (-6%), reduced pup weight and body weight gain, reduced brain weight, delay in vaginal patency, and decrease in cerebellar thickness.	B.6.6.2.1.2/01
Developmental neurotoxicity (DNT), perinatal dosing, gavage, dams	0, 6, 20, 60	Maternal 20 Developmental 20	Maternal 60 Developmental 60	Developmental: Decreased pup viability and pup body weights, altered learning in the spatial	B.6.6.2.1.2/01

and pups Not GLP Not in accordance with OECD test guideline Tebuconazole, no information about batch number, 97.4 % Rat Sprague-Dawley rats (strain: Tac: N(SD)fBR)				cognitive task and a number of organ weight changes at the highest dose tested. Tendency towards decreased number of live pups in 60 mg/kg bw/d group on PND 0 (p=0.07). The number of dead pups per litter was significantly increased in the 60 mg/kg bw/d. At birth, the the pup weight was reduced in the high dose group. Maternal weight gain during pregnancy reduced from 87.8 g (control) to 74.0 g (60 mg/kg bw/d group). The reduced litter weight partly explains the reduced maternal weight gain.	
Developmental toxicity, dermal GLP OECD test guideline not specified Tebuconazole, batch 16012/86, 97.4 % Rat WISW (SPF Cph)	0, 100, 300, 1000	<u>Maternal and developmental:</u> 1000 (top dose)	N/A	No systemic effects recorded.	B.6.6.2.1.3/01
Developmental toxicity, dermal (limit test)	0, 1000	<u>Maternal and Developmental:</u> 1000 (top dose)	N/A	No systemic effects recorded.	B.6.6.2.1.3/02

GLP OECD test guideline no. 414 (1981) Tebuconazole, batch 816196048, 96.2 or 95.8 % Rat WIST HanIbm (SPF)					
Tebuconazole 98%, Developmental toxicity, oral gavage Not GLP Rat, Wistar	0, 50, 100 Exposure from GD7 to PND16, with investigation on GD21 and postnatally	Maternal: 50 Developmental: ND	Maternal: 100 Developmental: 50	Maternal : post-implantation loss and postnatal death, prolonged gestation and late gestation resorptions. Moreover, two dams in the 100 mg/kg group were unable to give birth due to dystocia. Developmental : alterations in testicular testosterone (50 and 100 mg/kg) and progesterone (100 mg/kg). Altered endocrine tissue development in the offspring with virilised females with longer AGD and feminized males with increased nipple retention on PD 13 at both 50 and 100 mg/kg.. A LOAEL of 50 mg/kg bw/d can be identified from this study based on effects on sex steroid hormone and increased nipple retention in the male offspring. Increased absolute fetal liver weight at 100 mg/kg bw/d.	Taxvig et al 2007

Tebuconazole 98%, Developmental toxicity, oral gavage Not GLP Rat Wistar	0, 50 exposure from GD7-21	Maternal: ND Developmental: ND	Maternal: ND Developmental: ND	Maternal: increased frequency of post-implantation loss and a decrease in estradiol in dams. Developmental: significant increase in testicular progesterone levels in male foetuses (possible indicator of demasculinization of male foetuses).	Taxvig et al 2008
Tebuconazole 98.5%, Developmental toxicity, oral gavage Not GLP	0, 12,5, 50 from GD7 to PND16	Maternal: 50 Developmental: ND	Maternal: ND Developmental: 12.5	Maternal : none Developmental : at 12.5 and 50 mg/kg bw/d: females: ↑ AGD (PND1) At 50 mg/kg bw/d: males: ↑ nipple retention.	Hass et al 2012 Postnatal effects also reported in Jacobsen et al 2013 & Overgaard et al 2013
Developmental Toxicity: Rabbit					
Developmental toxicity, oral gavage GLP Comparable to OECD test guideline no. 414 (1981) Tebuconazole, batch 16007/83, 93.4 % Rabbit Himalayan CHBB:HM	0, 3, 10, 30	Maternal: 10 Developmental: 10	Maternal: >30 Developmental: 30	Maternal: Decreased body weight gain. Developmental: Increased resorptions.	B.6.6.2.2.1/01
Developmental toxicity, oral gavage GLP OECD test guideline no. 414 (1981) Tebuconazole, batch 16002/85,	0, 10, 30, 100	Maternal and developmental: 30	Maternal and developmental: 100	Maternal: Decreased food consumption and reduced body weight. Developmental: Increased post-implantation losses and an increase in malformations and anomalies.	B.6.6.2.2.1/02

98.2 % Rabbit Chinchilla rabbits, CHIN, hybrids, SPF quality					
Developmental toxicity, oral gavage GLP Comparable to OECD test guideline no. 414 (1981) Tebuconazole, batch 816196048, 96.3 - 96.8 % Rabbit Chinchilla rabbits (CHbb: CH, Hybrids, SPF quality)	0, 10, 30, 100	Maternal: 30 Developmental: 10 NOAEL of ~~30~~ 10 for developmental toxicity as agreed in previous RAR.	Maternal: 100 Developmental: 30	Maternal: Decreased food consumption and body weight gain. Developmental: Increased post-implantation loss, reduced foetal weight and increased incidence of malformations.	B.6.6.2.2.1/03
Developmental toxicity, oral gavage GLP Comparable to OECD test guideline no. 414 (1981) Tebuconazole, batch 278679012, 98.5 % Rabbit CHB-W (chinchilla) rabbits	0, 100	Maternal and developmental: N/A - only one dose tested.	Maternal and developmental: N/A - only one dose tested.	Maternal: reduced food consumption, decrease in overall corrected bw gain in dams however not statistically significant in dams (weight loss (day 6-10 p.c. only). Developmental: statistically significantly decreased foetal weight	B.6.6.2.2.1/04
Developmental Toxicity: Mouse					
Developmental toxicity, oral gavage GLP OECD test guideline no. 414 (1981) Tebuconazole batch	0, 10, 30, 100	Maternal: 100 (top dose) Developmental: 10	Maternal: N/A – no maternal toxicity recorded at the top dose. Developmental: 30	Maternal: N/A – no maternal toxicity recorded at the top dose. Developmental: Increased number of runts.	B.6.6.2.3.1/01

1616002/84, 93.6% (+ 5.5% symmetric isomer) Mouse NMRI/ORIG Kisslegg					
Developmental toxicity, oral gavage GLP OECD test guideline no. 414 (1981) Tebuconazole, batch 16012/86, 97.4 % Mouse NMRI/ORIG Kisslegg	0, 10, 20, 30, 100	Not reliable for setting NOAEL (small group size)	Not reliable for setting NOAEL (small group size)	<u>Maternal toxicity:</u> Decreased body weight gain, increased liver weights and associated histopathological changes.	B.6.6.2.3.1/02
Developmental toxicity, oral gavage GLP OECD test guideline no. 414 (1981) Tebuconazole, batch 816196048, 95.8 - 96.8 % Mouse NMRI KFM-HAN (outbred, SPF quality)	Main study: 0, 10, 30, 100 Supplementary study: 0, 1, 3	<u>Maternal:</u> 100 <u>Developmental:</u> -	<u>Maternal:</u> - <u>Developmental:</u> 10	<u>Maternal:</u> Liver effects seen at 30 and 100 mg/kg bw/d were considered adaptive. No adverse effects at any dose. <u>Developmental:</u> Total incidence of malformations (open eye, runts, cleft palate) was increased at the low dose of 10 mg/kg bw/d. *Both agreed at PRAPeR Expert Meeting 49 (2-6 June 2008)*	B.6.6.2.3.1/03
Developmental toxicity, dermal GLP OECD test guideline not specified Tebuconazole, batches	0, 100, 300, 1000	<u>Maternal:</u> 100 <u>Developmental:</u> 300	<u>Maternal:</u> 300 <u>Developmental:</u> 1000 (top dose)	<u>Maternal:</u> Liver toxicity (fatty changes and induction of mixed-function oxidase activities) <u>Developmental:</u> Increased incidence of cleft palate and	B.6.6.2.2.1/02

16002/85, 98.1 % and 816896061, 96.1 % Mouse NMRI KFM-HAN mice (Outbred SPF Quality)				supernumerary ribs.	

Reproductive performance and fertility as assessed and summarised by UK-RMS
The potential for tebuconazole to cause adverse effects on fertility, reproductive performance and pup survival, in addition to growth and development was investigated in a two generation reproductive toxicity study in the rat at levels of 100 – 1000 ppm in the diet.

No effects on fertility and reproductive performance were seen up to the top dose of 1000 ppm (approximately 72 - 111 mg/kg bw/d) at which parental and offspring toxicity occurred. The NOAEL for reproductive toxicity was therefore 72 mg/kg bw/d. Based on these findings, classification of tebuconazole under the CLP Regulation for effects on fertility is not warranted.

In adult parental animals, decreases in food consumption, retarded body weight gains and reduced organ weights were seen at 1000 ppm; a NOAEL for parental toxicity of 300 ppm (21.6 - 27.1 mg/kg bw in males and 27.8 - 33.9 mg/kg bw in females), at which no effects were seen, was therefore identified. In offspring, there were reduced body weight gains at 1000 ppm. A NOAEL for offspring toxicity of 300 ppm (21.6 - 27.1 mg/kg bw in males and 27.8 - 33.9 mg/kg bw in females) was established, with no effects observed at this level.

B.6.6.4 Overall summary on reproductive performace and fertility by DK-RMS:	***Reproductive performance and fertility as assessed and summarised by DK-RMS*** The main adverse effects of tebuconazole identified by the DK-RMS as relevant for classification for reproductive performance and fertility are 1) dystocia and prolonged gestation, 2) postimplantation loss, and 3) effects on the reproductive system of perinatally exposed males. Usually, in an evaluation of reproductive performance and fertility it is relevant to also include information from repeated dose studies investigating weight and histopathology of reproductive organs. In the present case however, for all three main adverse effects, results from repeated dose studies would be of limited relevance, and have therefore not been included in the present evaluation. Indications of endocrine disruption in repeated dose studies and targeted endocrine studies are supportive evidence for the mode of action behind the observed effects, and are presented in more detail in annex II. An overview of observed effects in rats, rabbits and mice is presented below. The potential for tebuconazole to cause adverse effects on fertility, reproductive performance and pup survival, in addition to growth and development was investigated in a two generation reproductive toxicity rat study (OECD TG 416, version 1983) at levels of 100 – 1000 ppm (up to max. 111 mg/kg bw/day) in the diet (B.6.6.1.1/01). In adult parental animals, slightly retarded body weight gains (<10% for most time points) were seen at 1000 ppm; In the first generation, litter size was reduced, indicating possible postimplantation loss. A NOAEL for parental toxicity of 300 ppm (22-34 mg/kg bw/d), at which no effects were seen, was therefore identified. In offspring, there were reduced body weight gains at 1000 ppm and reduced survival. A NOAEL for offspring toxicity of 300 ppm (22-34mg/kg bw/d) was established, with no effects observed at this level. In addition, several published studies using pre- and postnatal exposure were considered as supporting evidence, and are briefly presented endpoint by endpoint below. These studies were not compliant with OECD guidelines or GLP, but included relevant toxicity

	targets, some of which were not included in the submitted OECD and GLP compliant studies. **Dystocia and prolonged gestation:** Increased gestational length was seen in the highest tested dose in two rat studies, a US EPA guideline developmental neurotoxicity study (B.6.6.2.1.2/01), showing effects at a dietary dose of 65.9 mg/kg bw/day during gestation in SD rats and a published reproductive toxicity study (Taxvig et al 2007) showing effects at 50 & 100 mg/kg bw/day dosed as oral gavage in Wistar rats. No change in gestation length was seen at the lower doses in these two studies or in another published developmental study in Wistar rats tested up to 50 mg/kg bw/day (Hass et al. 2012) or at the highest dietary dose of 1000 ppm in the two-generation study B.6.6.1.1/01 in Wistar rats. This dose is listed as 95 and 111 mg/kg bw day in F0 and F1 females before mating, respectively In the two studies affecting gestation length, reductions in maternal body weight was seen during gestation, but changes in maternal body weight are generally not considered to influence gestation length, as determined from studies on feed restriction (Carney et al. 2004). Dystocia was seen in several studies. In the two-generation study (B.6.6.1.1/01), a death of one dam (F0) in the 1000 ppm group was possibly related to dystocia. This dam was found moribund. When sacrificed there were foetuses in both uterine horns, and the placentas in one horn were found to be very thick, beige coloured and hard. In B.6.6.2.1.2/01 two maternal deaths/moribund sacrifices (GD 22 or 23) at 1000 ppm corresponding to 66-125 mg/kg bw/day were related to dystocia. This was also observed in published study by Taxvig *et al.* 2007 (Wistar rats), two dams in the 100 mg/kg bw/d group were unable to give birth due to dystocia. These effects are consistent with adverse effects on pregnancy due to alterations of steroid hormones, which was also shown in the latter study. In particular, the seven-fold increase in late-gestation progesterone levels seen in the latter study is likely causative of dystocia. The reductions in maternal body weight gain during pregnancy is not considered to influence the ability to give birth, and effects are thus not secondary to systemic toxicity. This information on dystocia and prolonged gestation is considered relevant, and sufficient for classification for fertility. Even though the two-generation study showed no changes in gestation length and dystocia in only one dam, the observations in the US EPA DNT study and in the published studies serve as substantial evidence for these effects. In particular, the DNT study B.6.6.2.1.2/01 used 25 mated females per dose group and the same dietary dose of tebuconazole as the 2-generation study B.6.6.2.1.2/01. This may indicate strain differences. The findings by Taxvig et al. 2007 may indicate that effects of gavage exposure is more marked in these Wistar rats than seen with dietary exposure in the two-generation study also in Wistar rats. The relevance of these findings is strengthened by the occurrence of similar effects with other azole fungicides as discussed in section on sexual function and fertility. **Postimplantation loss/perinatal death:** See section 'B.6.6.4 Overall summary on developmental toxicity by RMS-DK'**.** **Effects on the reproductive system of perinatally/pubertally exposed male rats:** Tebuconazole does not appear to significantly affect male pup anogenital distance. Two studies investigated nipple retention (NR) in male offspring PD 13 and both found dose-dependent increased NR from 50 mg/kg bw/d (Taxvig et al. 2007, Hass et al. 2008). Sperm motility was not affected in two studies from the open literature investigating sperm in adult offspring after developmental exposure to tebuconazole in doses up to 50 mg/kg bw/d (Taxvig et al. 2007, Jacobsen et al., 2012). Sperm count was also not affected in two studies from the open literature investigating sperm counts in adult offspring after developmental (Taxvig et a. 2007) and developmental and pubertal exposure up to 60 mg/kg bw/d (B.6.6.2.1.2/01). Changes in weights and histology of male reproductive organs (epididymis, LABC, prostate, seminal vesicle, testis) were seen following perinatal or pubertal exposure in some studies (B.6.8.3.1.2/01, B.6.6.2.1.2/01).

	Overall, these effects on nipple retention and male reproductive organs may be relevant for classification for fertility, however a specific comparison with CLP criteria is needed to conclude on whether they are sufficient for classification. Effects on female AGD and AGDI, are consistent showing increases in 3 out of 4 studies, but the relevance of these findings for classification is unclear.

Developmental toxicity summarised by the UK-RMS

The potential for tebuconazole to adversely affect development was investigated in several regulatory developmental toxicity studies (the majority with compliant with or comparable to relevant OECD test guidelines), in the rat, rabbit and mouse.

Rat

The potential developmental toxicity of tebuconazole was investigated in the rat in seven studies; two standard guideline oral developmental toxicity studies, one new investigative (not to OECD test guideline) maternal toxicity study, two oral developmental neurotoxicity studies (not to OECD test guideline) and two studies via the dermal route (conducted to OECD test guidelines).

Two oral gavage studies were conducted, spanning doses of 10 -120 mg/kg bw/d up to day 15 post mating. Effects on development (increased incidence of total malformations and microphthalmia, post-implantation loss and decreased foetal weight) were seen at 100 mg/kg bw/d and above in presence of significant maternal toxicity (reduction in body weight gain). Signs of maternal toxicity varied between studies, with LOAELs of 30 and 60 mg/kg bw/d seen across the two studies. NOAELs of 30 and 60 mg/kg bw/d were identified for developmental toxicity across the two studies, reflecting differences in study design (i.e. different doses), even in presence of maternal toxicity. An overall NOAEL of 10 mg/kg bw/d was identified for maternal toxicity. A third study investigating maternal toxicity in more detail showed that the dose of 120 mg/kg bw/d caused severe maternal effects, including reduced body weight gain, decreased food consumption, clinical signs of toxicity, liver and adrenal toxicity.

In a regulatory dietary DNT study, developmental toxicity (pup mortality, reduced number of live born, reduced viability index, delayed vaginal patency, reduced body weight gain, reduced brain weight and decreased cerebellum thickness) was observed at the top dose of 65 - 125 mg/kg bw/d in the presence of significant maternal toxicity (mortality, reduced body weight gain and food consumption and prolonged gestation). Based on these findings, a NOAEL of 22 - 41 mg/kg bw/d was identified for both developmental and maternal toxicity from this study. However, tebuconazole did not cause any specific developmental neurotoxicity in the offspring when administered to the dams during gestation and lactation at dietary concentrations up to and including 125 mg/kg bw/d (the top dose).

A published DNT study is considered unreliable by the UK RMS.

In the two dermal developmental toxicity studies, there was no maternal or developmental toxicity up to the top dose of 1000 mg/kg bw/d.

Overall, developmental toxicity (pup mortality, reduced number of live born) was seen in rats from approximately 65 mg/kg bw/d (DNT study), increasing in severity (skeletal anomalies and increased incidence of total external malformations and microphthalmia) at around 100 - 120 mg/kg bw/d. The overall NOAEL for developmental toxicity in rats was 30 mg/kg bw/d. The observed developmental toxicity was always associated with significant maternal toxicity and it is possible that some of these developmental effects were the secondary unspecific consequence of maternal toxicity. An overall NOAEL of 10 mg/kg bw/d was identified for maternal toxicity in rats.

Rabbit

The developmental toxicity of tebuconazole was investigated in the rabbit in four studies, using two different strains (Himalayan and Chinchilla rabbits). Three of the four studies were conducted according to GLP and OECD test guidelines; the fourth study was a non-standard study investigating maternal toxicity in more detail.

Developmental toxicity (increased resorptions) started to occur from 30 mg/kg bw/d. At 100 mg/kg bw/d there was also decreased foetal weight and a slightly increased incidence of external malformations, including cleft palate, malrotation of hind limb, hemimelia and agenesis of claws. An overall NOAEL of 10 mg/kg bw/d was identified for developmental toxicity.

Maternal toxicity (decreased body weight gain) also started to occur from 30 mg/kg bw/d, becoming more severe (body weight loss, decreased food consumption, liver and adrenal hypertrophy and increased levels of corticosteroid hormones) at 100 mg/kg bw/d. An overall NOAEL of 10 mg/kg bw/d was also identified for maternal toxicity. It is possible that some of the developmental effects caused by tebuconazole in the rabbit were secondary to the observed maternal toxicity.

Mouse
It is possible that some of the developmental effects observed in the mouse were the secondary unspecific consequence of maternal toxicity.

The developmental toxicity potential of tebuconazole was investigated in the mouse in four guideline studies; three by oral administration (one of which was limited to an investigative study of maternal toxicity) and one by dermal administration.

In the oral studies, developmental toxicity (small foetuses (runts), increased incidence of post-implantation loss and delayed ossification) started to occur from 30 mg/kg bw/d, becoming more severe (reduced foetal weight and slightly increased incidence of external malformations such as cleft palate, exencephaly and tail abnormalities) at 100 mg/kg bw/d. The RMS considers an overall NOAEL of 10 mg/kg bw/d can be identified for developmental toxicity in the mouse as no effects were seen at this dose.

Maternal toxicity (liver toxicity) also started to occur from 30 mg/kg bw/d, becoming more severe (haematotoxicity, decreased body weight gain, reduced food consumption) at 100 mg/kg bw/d. Therefore, the RMS considers an overall NOAEL of 10 mg/kg bw/d can be identified for maternal toxicity in the mouse. Although the NOAELs above have been identified, the RMS notes that in PRAPeR Expert Meeting 49 (2-6 June 2008) the following NOAELs were agreed: the maternal NOAEL was set at 100 mg/kg bw/d – since liver effects were considered by the experts as adaptive but not adverse, and the developmental LOAEL was set at 10 mg/kg bw/d – based on an increased incidence of malformations (open eye, runts, cleft palate). The RMS accepts the decision made at the PRAPeR meeting and the LOAEL of 10 mg/kg bw/d will be taken forward into the risk assessment.

In the dermal study, increased incidences of cleft palate and supernumerary ribs were seen at top dose of 1000 mg/kg bw/d, at which maternal toxicity (liver toxicity) also occurred. Therefore, a NOAEL of 100 mg/kg bw/d was identified for maternal toxicity and a NOAEL of 300 mg/kg bw/d was identified for developmental toxicity from this study.

Publications
There are also eight publications (three *in vitro* investigations and five *in vivo* targeted developmental toxicity studies in rats) of potential relevance to developmental toxicity. Some of these involve *in vitro* investigations and are of limited relevance. The limited *in vivo* investigations, all in the rat and from the same research group tend to show increased post-implantation loss and effects on sex steroid hormone levels in foetuses/pups and dams from around 50 mg/kg bw/d. An overall LOAEL of 50 mg/kg bw/d can be identified from these publications, which is consistent with the overall developmental toxicity profile from the standard regulatory studies and the NOAEL of 30 mg/kg bw/d for the rat identified from these.

Overall
Overall, developmental toxicity (slightly increased incidence of external malformations, post-implantation loss, reduced ossification, decreased foetal weight, skeletal anomalies, pup mortality, reduced viability index) was observed in rats, rabbits and mice. The observed developmental effects were frequently associated with significant maternal toxicity (effects on body weights, liver and adrenal toxicity). It is therefore possible that some of the developmental effects observed in the three species investigated were the secondary unspecific consequence of maternal toxicity. On this basis, the current harmonised classification of tebuconazole for developmental toxicity in category 2 (Repr 2; H361d) is still appropriate. **The lowest NOAEL for both maternal and developmental toxicity frequently seen across all development toxicity studies was 10 mg/kg bw/d, identified in both the rabbit and the mouse. However, it is noted that a LOAEL of 10 mg/kg bw/d for developmental toxicity was**

agreed at PRAPeR Expert Meeting 49 (2-6 June 2008).

B.6.6.4 Overall summary on developmental toxicity by DK-RMS:	***Developmental toxicity*** The potential for tebuconazole to adversely affect development was investigated in several regulatory developmental toxicity studies (the majority in compliance or comparable to relevant OECD test guidelines), in the rat, rabbit and mouse. There are also eight publications (three *in vitro* investigations and five *in vivo* targeted developmental toxicity studies in rats) of potential relevance to developmental toxicity. Three main adverse developmental effects of tebuconazole are considered as critical for the classification on developmental toxicity : 1) postimplantation loss and perinatal death, 2) fetal/pup growth impairment, and 3) external malformations including cleft palates. An overview of observed effects is presented here, followed by more detailed information on studies in rats, rabbits and mice. **Developmental effect overview** 1) Developmental exposure to tebuconazole, typically at doses above 50 mg/kg bw/day, clearly and consistently affects fetal and postnatal survival. In some but not all studies, reduced maternal weight gain is seen at the doses causing fetal death. Reduced maternal weight gain may result from systemic toxicity or from endocrine disruption of pregnancy, including reduced growth of offspring and fetal death. . For every study, these issues need to be addressed, and a case-by-case evaluation is necessary to evaluate whether the observed maternal weight changes may be a cause of the adverse effects including fetal death or not. If the maternal effects can be considered mild compared to the developmental effects, these cannot be explained as secondary to maternal toxicity. This is also supported by two feed restriction studies on the rat and rabbit (Fleeman, 2005 and Cappon, 2005), which clearly showed that severe weight loss or decrease in body weight gain induced minor changes in skeleton development but had no effects on viability or malformations in the rat. Ten regulatory studies using oral exposure route, report significant increase in embryonic/fetal death or post-implantation loss, or reduced litter size (B.6.6.1.1/01, B.6.6.2.1.2/01, B.6.6.2.1.1/01, B.6.6.2.1.1/02, B.6.6.2.1.1/03, B.6.6.2.2.1/01, B.6.6.2.2.1/02; B.6.6.2.1.3/02, B.6.6.2.2.1/03 and B.6.6.2.3.1/03). Importantly, several studies included doses where fetal death was observed with no or only minor reduction of maternal body weight gain. Specifically, B.6.6.1.1/01 (ID 17) identified reduced litter size in a rat two-generation study (statistically significant at first mating), at a dose not affecting maternal body weight. A mouse developmental toxicity study showed significant post-implantation loss and reduced litter size without effect on maternal body weight gain (B.6.6.2.3.1/03, ID 29). In a rat developmental toxicity study, reduced postimplantation loss/increased number of resorptions was seen at a dose of 120 mg/kg bw/d which also reduced maternal body weight gain, but this reduced weight gain could be fully explained by smaller total litter weight (B.6.6.2.1.1/02, ID 19). In addition, B.6.6.2.1.2/01 (ID31) showed slightly reduced number of live born pups and reduced viability index, at a dietary dose (up to 65 mg/kg bw/d during gestation) causing slightly reduced maternal body weight gain and food consumption both not considered to be treatment related due to no dose-dependency. B.6.6.2.3.1/01 (ID 27), reported no significant effect on fetal death at doses up to 100 mg/kg bw/d, but data indicates increased numbers of resorptions as well as post-implantation loss in the high dose group without any changes in maternal body weight gain. In a few of the rat studies the doses causing fetal death (120 and 100 mg/kg bw/d) also caused reduced maternal weight gain which could not fully be explained by a lower litter weight (B.6.6.2.1.1/03, , ID 20; B.6.6.2.1.1/01, ID 18). In rabbits, the fetal death was often seen at doses also reducing maternal body weight gain (B.6.6.2.2.1/01, B.6.6.2.2.1/02, B.6.6.2.2.1/03 In the open literature additional studies showed increased post implantation loss at 50 mg/kg bw/d in a developmental study (Taxvig et al. 2008, ID 53) not showing any change in maternal body weight gain, and at 100 mg/kg bw/d in a perinatal study (Taxvig et al.

	2007, ID 54) showing reduced maternal body weight gain during pregnancy, but no significant change in adjusted body weight change (bw minus uterine weight at caesarean section). No effect was observed in one open literature study at doses up to 50 mg/kg bw/d (Hass et al. 2012, ID 52) and in one rabbit study at 100 mg/kg bw/d (B.6.6.2.2.1/04, ID 26). Additionally B.6.6.2.1.2/01 (ID 55) report tendencies or nominal effects on litter size at the highest doses tested (60 mg/kg bw/d), though not statistically significant. Studies using dermal exposure route (B.6.6.2.1.3/02, B.6.6.2.1.1/01, B.6.6.2.3.2/01) report no effects on fetal death/reduced litter size. In studies with continued exposure during the lactation period, postnatal offspring survival is also adversely affected by tebuconazole, as two studies show a clearly decreased litter viability (B.6.6.2.1.2/01, ID 31; B.6.6.2.1.2/01, ID 55). 2) There is clear and consistent evidence that oral exposure to tebuconazole causes growth impairment in both fetuses and pups. Nine out of 14 developmental toxicity studies show reduced offspring weights (B.6.6.1.1/01, B.6.6.2.1.1/01, B.6.6.2.1.1/02, B.6.6.2.1.1/03, B.6.6.2.2.1/02, B.6.6.2.2.1/03, B.6.6.2.2.1/04, B.6.6.2.3.1/01, B.6.6.2.3.1/03), and the studies which do not show this had either used dermal exposure route (B.6.6.2.1.3/02, B.6.6.2.1.1/01, B.6.6.2.3.2/01) or an exposure of 50 mg/kg bw/d or below (B.6.6.2.2.1/01, B.6.6.3/08), which seems to be an approximate threshold for effect on this endpoint. In addition, effects were seen across species (rat, rabbit and mice) confirming the growth retardation effect of tebuconazole exposure. In rabbits, B.6.6.2.2.1/02 observed a marginal decreased fetal body weight (6 % change compared to control) was seen, which correlated with slightly retarded ossification. As described for fetal death/postimplantation loss, not all studies showed changes in maternal weight gain at effective doses, and in some studies the reduced maternal weight gain could be explained by smaller litter weight. 3) There is also evidence that oral exposure to tebuconazole causes external malformations including cleft palates in several studies. In rats developmental effects such as skeletal anomalies and increased incidence of total external malformations and microphthalmia was seen in two developmental toxicity studies using higher doses of around 100 - 120 mg/kg bw/d (B.6.6.2.1.1/01, B.6.6.2.1.1/02). Presence of cleft palate was rare in these studies and not related to exposure. In one study on dermal toxicity, one case was seen in a control group (B.6.6.2.1.1/01). In rabbits, B.6.6.2.2.1/02 observed a slightly increased incidence of external malformations, including cleft palate, malrotation of hind limb, enlarged fontanelle, hemimelia and agenesis of claws at 100 mg/kg bw/d (total incidence of 33.3 compared to 0 in control). Cleft palate was seen in 1.1% of the fetuses in 6.7% of the litters at the high dose. In oral studies in mice, developmental toxicity (delayed ossification) started to occur from 30 mg/kg bw/d, becoming more severe (reduced foetal weight and slightly increased incidence of external malformations such as cleft palate, exencephaly and tail abnormalities) at 100 mg/kg bw/d. In B.6.6.2.3.1/01 the number of foetuses with cleft palate was increased (> 10 % change compared to control). Cleft palate is a common malformation in this strain of mice. However, incidence at 100 mg/kg bw/day were outside of the range of the HCD provided (Table 53 of the CLH report). Other malformations and anomalies (face malformations, kinked and shortened tail, dilation of brain ventricles, vertebral asymmetry, spinal dysplasia, rib fusion, partial aplasia of parietal bone) were also increased above controls and historical control data (> 10 % change compared to control at 100 mg/kg bw/day). In B.6.6.2.3.1/03 the total incidence of malformations (open eye, runts, cleft palate) was increased already at the low dose of 10 mg/kg bw/d. B.6.6.2.3.2/01 showed in a dermal study in mice an increased incidence of cleft palate and supernumerary ribs at 1000 mg/kg bw/day. Malformations were seen across species (rat, rabbit and mice), cleft palates only in mice and rabbits confirming effect of tebuconazole exposure. **Study overview by species** **Rat**

	The potential developmental toxicity of tebuconazole was investigated in two standard guideline oral developmental toxicity studies, one new investigative (not to OECD test guideline) maternal toxicity study, two oral developmental neurotoxicity studies (not to OECD test guideline) and two studies via the dermal route (conducted to OECD test guidelines), and three published studies. In addition, the two-generation study in rats described above is relevant for evaluation of developmental toxicity. Two oral gavage studies were conducted, spanning doses of 10 -120 mg/kg bw/d up to day 15 post mating (B.6.6.2.1.1/01, B.6.6.2.1.1/02). Effects on development (increased incidence of total malformations and microphthalmia, post-implantation loss and decreased foetal weight) were seen at LOAELs of 100 and 120 mg/kg bw/d in presence of mild maternal toxicity (reduction in maternal body weight gain). Differences in NOAELs of 30 and 60 mg/kg bw/d for developmental toxicity reflected differences in study design (i.e. different doses) in the two studies. Effects on maternal body weight gain was only seen during treatment (GD 6 to 15), and varied between studies, with LOAELs of 30 and 60 mg/kg bw/d for 15-16% reduction compared to control (NOAELs 10 and 30 mg/kg bw/d). At higher doses (100 and 120 mg/kg bw/d), the reduction in maternal body weight gain could be partly or fully attributed to lower litter weights due to reduced litter size and reduced fetal weight. A third study (B.6.6.2.1.1/03) investigating maternal toxicity at high dose in more detail at GD 16 and showed that the applied dose of 120 mg/kg bw/d caused marked maternal effects, including reduced body weight gain (reduction to 62% of control value at GD 16), decreased food consumption (reduced to 82% of control at GD 16), clinical signs of toxicity (piloerection), and changes in relative but not absolute liver and adrenal weights. Fetal weight was reduced to 74% of control weights. The reductions in maternal body weight gain may be related to reduced food intake during early days of pregnancy and/or to specific toxicity to uterine and fetal growth. Therefore, a thorough evaluation of relationships between maternal weight gain changes and fetal growth and survivial needs to be carried out. This analysis is presented for each study in Vol 3. See also section " Considerations regarding presence or absence of marked systemic effects" below. In general, reductions in maternal body weight gain in high dose groups were largest (in percent of control) in the first days of exposure. At termination of the studies, reduced fetal weights and reduced litter sizes caused reduced total litter weights, which could partly or fully explain the differences in maternal body weight gain during pregnancy indicating absence of marked systemic maternal effect according to CLH criteria Annex I: 3.7.2.4.4. In the B.6.6.2.1.1/03 study, examinations were carried out already at GD 16, and the litter weight differences were smaller than seen in the other studies at GD 20-21 (B.6.6.2.1.1/01, B.6.6.2.1.1/02) when reduced litter weights could largely explain the observed reductions in maternal body weight gain. In a regulatory dietary DNT study (B.6.6.2.1.2/01), developmental toxicity (pup mortality, reduced number of live born, reduced viability index, delayed vaginal patency, reduced body weight gain, reduced brain weight and decreased cerebellum thickness) was observed at the top dose of 65 - 125 mg/kg bw/d in the presence of mild maternal toxicity (reduced body weight gain (16% reduction) and food consumption (5% reduction) and prolonged gestation). Observations of two dead dams in this group are related to parturition problems (dystocia is probable cause of death according to study report). The prolonged gestation and dystocia can be considered a specific effect of the substance rather than unspecific maternal toxicity, as this is related to endocrine disruption (see Section 2.10). The number of dams with stillborn pups was slightly increased from 2 dams in control group to 5 dams in high dose group, but the number of stillborn pups was higher (2 in control and 7 in high dose group). After birth, the viability index (live pups at day 5 divided by live born pups) was significantly reduced. Based on these findings, a NOAEL of 22 - 41 mg/kg bw/d was identified for both developmental and maternal toxicity from this study. In a two-generation study (B.6.6.1.1/01), retarded weight gains for parents and pups were seen at 1000 ppm (72 - 111 mg/kg bw/d). In mating of the F0 generation (both in cohort a and b) there were statistically significant effects showing a higher number of stillborn pups,

	lower viability index (i.e. pup survival from birth to PND 4), lower lactation index (i.e. pup survival from PND4-21), and a lower litter size, which could be related to postimplantation loss. In the F1 generation a dose of 1000 ppm resulted in markedly increased offspring mortality and moderately reduced offspring growth, whereas in the F2 generation there was no increase in offspring mortality but even more marked reductions in postnatal offspring growth. One dam of the 1000 ppm group died of endometritis (secondary to dystocia). Generally, the body weight reduction seen in the offspring were more marked that the corresponding reductions on maternal weight during the lactation period (5-11% decrease), indicating that tebuconazol caused specific developmental toxicity effects in the offspring. DK-RMS considers it unlikely that all of the adverse effects observed in the offspring were unspecific consequences, secondary to maternal toxicity. DK-RMS considers the NOAEL for reproductive toxicity in this study to be 300 ppm, and not 1000 ppm as suggested by the UK-RMS. This is consistent with a previously agreed NOAEL for reproductive toxicity and also consistent with the reproductive NOAEL provided in the study report for this 2-generation study. A published DNT study (B.6.6.2.1.2/01) is considered unreliable by the UK RMS, but reliable with restrictions by DK RMS. Withdrawal of neuropathological findings does not question the validity of the reproductive toxicity data. At the top dose of 60 mg/kg bw/d, maternal body weight gain was significantly decreased, postimplantation loss was significantly increased, and pup birth weight was significantly decreased. In addition, this study also showed reduced pup viability (2.2 vs 0.4 dead per litter in control group). The reduced maternal body weight gain (13.8 g) can likely be explained by smaller litter weight (9.6 g) and assumed proportionally smaller uterine and amniotic fluid weights. The observed effects on postimplantation loss and pup viability are thus not attributed to maternal toxicity. In the two dermal developmental toxicity studies, there was no maternal or developmental toxicity up to the top dose of 1000 mg/kg bw/d (B.6.6.2.1.3/01 and B.6.6.2.3.1/03). It is noted that these doses were applied to skin for 6 hours per day, and no evaluation of internal dose was performed. The lack of effects in these studies may be due to lower internal doses using this exposure route compared to oral exposure. Three published studies using perinatal exposure of rats were published by the same group. One study showed reduced maternal body weight at GD 21, increased postimplantation loss and reduced pup birth weight at 100 mg/kg bw/d (B.6.6.3/07). This and two similar studies carried out in the same lab showed no or minor changes in maternal body weight gain or pup birth weight at 50 mg/kg bw/day (B.6.6.3/04, B.6.6.3/07, B.6.6.3/08). Postimplanation loss was seen at 50 mg/kg bw/d in two of the three studies. In the first application for approval, the UK_RMS concluded: « *An overall LOAEL of 50 mg/kg bw/d can be identified from these publications, which is consistent with the overall developmental toxicity profile from the standard regulatory studies and the NOAEL of 30 mg/kg bw/d for the rat identified from these* ». Overall, developmental toxicity (pup mortality, reduced number of live born) was seen in rats from approximately 50-65 mg/kg bw/d (two DNT studies and two of three published perinatal exposure studies). Developmental effects increased in severity (postimplantation loss and reduced fetal weights, skeletal anomalies and increased incidence of total external malformations and microphthalmia) in two developmental toxicity studies using higher doses of around 100 - 120 mg/kg bw/d. Presence of cleft palate was rare in these studies and not related to exposure (1 case in a control group, B.6.6.2.1.1/01). The overall NOAEL for developmental toxicity in rats was 30 mg/kg bw/d. In general the observed developmental toxicity was associated with changes in maternal body weight gain, particularly in the early part of exposure, but continuing throughout gestation. Notably, this reduced maternal body weight gain may be due to direct effects on uterine factors and fetal development. When comparing maternal weight gain during pregnancy with reductions in litter weight, it is important to note that maternal weight gain is dependent on several factors including weight of litters, uterus and amniotic fluid. The

developmental effects cannot by default be consided unspecific maternal toxicity. Further discussion in relation to CLP criteria is presented below.

Rabbit

The developmental toxicity of tebuconazole was investigated in the rabbit in four studies, using two different strains (Himalayan and Chinchilla rabbits). Three of the four studies were conducted according to GLP and OECD test guidelines; the fourth study was a non-standard study investigating maternal toxicity in more detail.

Developmental toxicity (increased resorptions) started to occur from 30 mg/kg bw/d (B.6.6.2.2.1/01, B.6.6.2.2.1/02, B.6.6.2.2.1/03). At 100 mg/kg bw/d there was also decreased foetal weight and a slightly increased incidence of external malformations, including cleft palate, malrotation of hind limb, hemimelia and agenesis of claws. B.6.6.2.2.1/02 observed a marginally decreased foetal body weight (6 % change compared to control), which correlated with slightly retarded ossification. In addition, an increased incidence of external malformations occurred at 100 mg/kg bw/day, including cleft palate, malrotation of hind limb, enlarged fontanelle, hemimelia and agenesis of claws (total incidence of 33.3 at 100 mg/kg bw/day compared to 0 in control). Cleft palate was seen in 1.1% of the fetuses in 6.7% of the litters at the high dose. A dose-response pattern was seen in the incidence of skeletal findings being statistically significant at 100 mg/kg bw/day, but starting at the low dose of 10 mg/kg bw/day.

In a study B.6.6.2.2.1/03 an increased incidence of malformations is seen at 30 mg/kg bw/day, but UK-RMS argues that this is due to higher background levels in the period of performing this study. DK RMS acknowlegdes the HCD data, but have reservations for using the data to dismiss the effects based on several observations described in the following; No increased incidence of malformations were seen in concurrent controls. In Table 2.6.6.2.1 HCD ranges of 0.0-0.9 % for foetus incidence and 0.0-7.7% for litter incidence for multiple malformation are given; this seems however to be based on 1 fetus affected in one litter in 3. study from Dec 91/Jan 92 containing 13 litters and 107 fetuses. None of the other 7 studies (or 9 if two other studies are included: 2. study nov 91/jan 92 and 1.study nov 92/jan 93) from 1992 each containing from 14-16 litters reported multiple malformation.
In the concurrent control group containing 16 litters and 141 foetuses or in the low dose group (10 mg/kg bw/d; 15 litters and 142 foetuses) no external or multiple malformations were reported. The concurrent control group is of comparable size with the HCD data driving the range of 0.0-7.7%. In the concurrent study at 30 mg/kg bw/d three foetuses from three different litters were affected (one fetus with malpositioned hind legs, one fetus with arthrogryposis, and one fetus with multiple malformations) which raises concerns. At 100 mg/kg bw/day three foetuses from three different litters had multiple malformations. Furthermore, it is also noted that it seems the HCD ranges in general have included the control group of the concurrent study which is inappropriate.
According to ECETOC (Monograph No. 31 2002) and Moore et al. 2013[1] runts are considered of high concern on their own and listed under external abnormalities and malformations. It could be discussed whether runts should be taken out of the external findings as proposed by applicant and accepted by UK-RMS in Table 52 of the CLH report. In consideration of the large historical control database, the individual specific external malformations seem to be rare spontaneous events and typically observed in 1 foetus in one litter. It could be argued that it would seem unlikely that 3 fetuses from 3 litters with external malformations arising as spontanoues events should then be detected in the current study at 30 mg/kg bw/d and also considering that treatment related malformations are seen in the highest dose. Statistical significance does not need to be present to validate the biological significance of treatment-related effects. This is particularly true of findings with low incidence (i.e., rare malformations) or high variability, or in situations

[1] **Moore et al., 2013. Guidance on classification for reproductive toxicity under the globally harmonized system of classification and labelling of chemicals (GHS). Crit Rev Toxicol, 2013; 43(10): 850–891.**

where the concurrent control data have an unusual incidence profile (OECD GD 43, 2008).
It was argued by UK-RMS that study B.6.6.2.2.1/01 gave no indications for a treatment-related effect on external malformations up to and including the highest tested dose of 30 mg/kg bw/day. However this study was of low reliability and was only accepted as supplementary information, due to the poor reporting, reduced database being available for inspection, doses tested being low (not tested up to maternal toxicity) and increased number of losses not being commented.

An overall NOAEL of 10 mg/kg bw/d was identified for developmental toxicity.
Maternal toxicity (decreased body weight gain) also started to occur from 30 mg/kg bw/d, becoming more severe (body weight loss, decreased food consumption, liver and adrenal hypertrophy and increased levels of corticosteroid hormones) at 100 mg/kg bw/d. An overall NOAEL of 10 mg/kg bw/d was also identified for maternal toxicity.
The developmental effects cannot by default be consided related to unspecific maternal toxicity. Further discussion on possible relations with maternal effects is presented below in relation to CLP criteria.

Mouse

The developmental toxicity potential of tebuconazole was investigated in the mouse in four guideline studies; three by oral administration (one of which was limited to an investigative study of maternal toxicity) and one by dermal administration.
In the oral studies, developmental toxicity (small foetuses (runts), increased incidence of post-implantation loss and delayed ossification) started to occur from 30 mg/kg bw/d (B.6.6.2.3.1/03), becoming more severe (reduced foetal weight, reduced litter size and slightly increased incidence of external malformations such as cleft palate, exencephaly and tail abnormalities) at 100 mg/kg bw/d (B.6.6.2.3.1/03 and B.6.6.2.3.1/01).
Cleft palate was seen in two mouse studies. In one study, the number of foetuses with cleft palate was increased (> 10 % change compared to control) (B.6.6.2.3.1/01). Cleft palate is a common malformation in this strain of mice, however incidence at 100 mg/kg bw/day were outside of the range of the HCD provided (table 53 of the CLH report). Other malformations and anomalies (face malformations, kinked and shortened tail, dilation of brain ventricles, vertebral asymmetry, spinal dysplasia, rib fusion, partial aplasia of parietal bone) were also increased above controls and historical control data (> 10 % change compared to control at 100 mg/kg bw/day).
The HCD coming only from the performing laboratory is considered more appropriate by DK-RMS. These data were obtained within a 5 year period centered around study B.6.6.2.3.1/01 study as well.

In another study (TG 414), the total incidence of malformations (open eye, runts, cleft palate) was increased already at the low dose of 10 mg/kg bw/d (B.6.6.2.3.1/03).

In a dermal study, an increased incidence of cleft palate and supernumerary ribs was seen at 1000 mg/kg bw/day (top dose) associated with liver effects in the dam (.6.6.2.3.2/01).

Maternal toxicity (liver toxicity) also started to occur from 30 mg/kg bw/d, becoming more severe (haematotoxicity, decreased body weight gain, reduced food consumption) at 100 mg/kg bw/d. In an earlier version of the RAR, the UK RMS based on these findings considered an overall NOAEL of 10 mg/kg bw/d for maternal toxicity in the mouse. The UK RMS noteed that in PRAPeR Expert Meeting 49 (2-6 June 2008) the following NOAELs were agreed: the maternal NOAEL was set at 100 mg/kg bw/d – since liver effects were considered by the experts as adaptive but not adverse, and the developmental LOAEL was set at 10 mg/kg bw/d – based on an increased incidence of malformations (open eye, runts, cleft palate) in the study B.6.6.2.3.1/03. The UK RMS accepted the decision made at the PRAPeR meeting and the LOAEL of 10 mg/kg bw/d was taken forward. The DK RMS agrees with this NOAEL for maternal effects and LOAEL for developmental effects (from PRAPeR Expert Meeting 49), however notes that effects on

	maternal body weight gain and altered liver weight and histology during pregnancy may be a direct and specific effect related to an endocrine mode of action, rather than an unspecific secondary effect of maternal toxicity. Thus, the observed developmental effects in mice cannot be considered related to unspecific maternal toxicity. Further discussion on possible relations with maternal effects is presented below in relation to CLP criteria.

B.6.7. NEUROTOXICTY

B.6.7.1. Neurotoxicity studies in rodents

The neurotoxicity of tebuconazole was investigated in multiple studies conducted via the oral route. An acute oral gavage study (B.6.7.1.1/01) and 90-day dietary study (B.6.7.1.2/01), owned by Bayer Task Force (BTF), were evaluated in the original DAR (2006), peer reviewed at EU level (EFSA Conclusion, 2008), and were considered acceptable. In addition, the EU Tebuconazole Task Force (EU TTF) has provided a new oral acute neurotoxicity study (B.6.7.1.1/02) for the purposes of renewal. The BTF has provided no new data on neurotoxicity in rodents. No delayed polyneuropathy studies have been submitted by either task force. An *in vitro* investigation of potential relevance to neurotoxicity has been identified from the literature.

B.6.7.1.1. *Acute oral neurotoxicity study in the rat*

1)

Previous evaluation:	In Tebuconazole DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.7.1.1/01
Study title	An acute oral neurotoxicity screening study with technical grade Tebuconazole (Folicur) in Fischer 344 rats Supplemental: A Special Acute Oral Neurotoxicity Study to Establish a No-Observed-Effect Level with Technical Grade Tebuconazole in Fischer 344 Rats
Test substance	Tebuconazole
Purity (%) **Batch no.**	96.2 - 97.3 Batch no.: 603-0013 Original and supplemental studies.
Test animals	Male and female Fischer 344 CDF(F-344)/BR, fasted.
Groups	12/sex/dose level
Dose	Main study 0 (vehicle), 100, 500 and 1000 mg/kg bw for males 0, 100, 250 and 500 mg/kg bw for females Single dose Supplementary study 0 (vehicle), 20 of 50 mg/kg bw (males and females) Single dose
Route	Oral (gavage)
Vehicle	0.5 % methylcellulose / 0.4 % Tween 80 in deionized water
GLP	Yes
Guideline	Similar to OECD Guideline 424 (1997)
Deviation	The following deviations from the OECD-Guideline 424 (1997) occurred: None
Acceptable	Acceptable
NOAEL	Neurotoxicity: 50 mg/kg bw (both sexes). Generalised toxicity: 50 mg/kg bw (both sexes).
Effects at the LOAEL	Neurotoxicity: based on FOB and motor activity. Generalised toxicity: based on clinical signs of toxicity.

Methods
Tebuconazole was administered orally by gavage to young Fischer 344 rats, using nominal concentrations selected based on a screening study of 0, 100, 500, 1000 mg/kg bw (males) and 0, 100, 250 and 500 mg/kg bw (females). The post-treatment period was 15 days. Analytical chemistry included analysis of feed and water, analysis of test substance and of test substance in the vehicle. Neurobehavioral evaluation was conducted on all 12 rats/sex/dose level. Clinical observations and body weight were performed on a weekly basis. Detailed examinations for clinical signs of toxicity were performed on a daily basis. Automated measurements of motor and locomotor activity (figure-eight maze) together with functional observational battery (FOB) were performed, together with brain weight determination, gross necropsy and histology. Skeletal muscle, peripheral nerves, eyes (with optic nerves)

and tissues from the central nervous system were also examined microscopically for lesions.

Evidence of neurotoxicity at the low dose (100 mg/kg bw) meant a supplementary study was conducted using doses 0, 20 and 50 mg/kg bw to establish a NOAEL. FOB was performed four hours after administration of the dose, with measurement of activity concluded at seven hours.

Results

Mortality

1 of 12 male rats died at 500 mg/kg bw and 6 of 12 male rats died at 1000 mg/kg bw. These males died within one or two days following treatment. No females died at any dose level (100, 250 or 500 mg/kg) prior to terminal sacrifice, 15 days following treatment.

There were no deaths prior to terminal sacrifice in the supplemental study.

Body weight

At 500 mg/kg bw body weight was reduced in eleven males. The lack of effect at 1000 mg/kg bw in males is ascribed to a selection bias for that group, with the most severely affected animals not surviving until day 7. Body weight was not affected in any females at any dose level.

Table 6.7-1. Body weight data

	Nominal Dose - Males			
	0 mg/kg	**100 mg/kg**	**500 mg/kg**	**1000 mg/kg**
Pre-treatment	156 ± 7	159 ± 10	155 ± 8	156 ± 4
Day 0[b]	163 ± 6	164 ± 9	159 ± 10	162 ± 4
Day 7	209 ±9	208 ± 13	197*[c] ± 12	200[d] ± 8
Day 14	232 ± 10	231 ± 15	223[c] ± 16	234[d] ± 6
	Nominal Dose - Females			
	0 mg/kg	**100 mg/kg**	**250 mg/kg**	**500 mg/kg**
Pre-treatment	123 ± 5	123 ± 4	123 ± 4	122 ± 4
Day 0[b]	118 ± 6	116 ± 3	117 ± 5	116 ± 3
Day 7	114 ± 6	142 ± 5	144 ± 6	142 ± 4
Day 14	155 ± 9	154 ± 6	155 ± 7	153 ± 7

[a]Mean ±S.D. for n=12 (except as noted)
[b]Fasted body weight measurements
[c]Mean ±S.D. for n=11 survivors
[d]Mean ±S.D. for n=6 survivors
*Significantly different from control at p≤ 0.05 (ANOVA)

Organ weight

There was no effect on brain weight. Brain weights were not affected by treatment at any dose level in females or surviving males at terminal sacrifice.

Clinical signs

At 500 and 1000 mg/kg bw uncoordinated gait, decreased activity, cool-to-touch body, salivation, clear lacrimation, various stains (urine, red nasal, red lacrimal and oral) were observed in males. At 250 and 500 mg/kg bw, uncoordinated gait and various stains (red nasal and oral stains) were observed in females. In general, the incidence of clinical signs increased with dose. Compound-related signs were apparent in both sexes on the day of treatment (day 0) and resolved by day 3 following treatment. The only remaining clinical sign, perianal stain, was evident in both sexes at all dose levels, including controls. This sign is attributed to exposure to the vehicle and is not related to treatment with tebuconazole.

Table 6.7-2. Clinical signs - main study

Signs	Nominal Dose - Males			
	0 mg/kg [a]	**100 mg/kg[a]**	**500 mg/kg[b]**	**1000 mg/kg[c]**
Uncoordinated Gait (Ataxia)	--[d]	--	9 (0)	8 (0-1)
Decreased Activity	--	--	2 (0-1)	10 (0-1)
Salivation	--	--	1 (1)	1 (1)
Cool to touch, body	--	--	1 (0-1)	6 (0-1)
Lacrimation , clear	--	--	1 (0-1)	5 (0-1)
Urine Stain	--	--	1 (0-1)	7 (0-2)
Nasal Stain, red	--	--	11 (0-1)	5 (0-1)
Oral Stain	--	--	2 (0-1)	4 (0-2)
Lacrimal Stain, red	--	--	1 (0-1)	1 (0-2)
Perianal Stain	6 (0-2)	8 (0-3)	11 (0-5)	9 (0-3)
Signs	**Nominal Dose - Females**			
	0 mg/kg[a]	**100 mg/kg[a]**	**250 mg/kg[a]**	**500 mg/kg[a]**
Uncoordinated Gait (Ataxia)	--[d]	--	6 (0)	12 (0-1)
Nasal Stain, red	--	--	11 (0-1)	12 (0-3)
Oral Stain	--	--	2 (0)	1 (0)
Lacrimal Stain, red	--	--	--	1 (0-1)
Perianal Stain	3 (0-1)	3 (0-2)	12 (0-2)	12 (0-3)

[a] Incidence for n=12 (days observed, except where noted otherwise.
[b] Incidence for n=11 (days observed), after day 1.
[c] Incidence for n=6 (days observed), after day 1.
[d] Not observed

Compound-related signs were not evident in males or females that received doses of 20 or 50 mg/kg bw in the supplemental study. Perianal stain was evident in one or two control or treated males as an incidental sign following treatment.

Motor and Locomotor Activity Testing
The figure-eight maze test showed effect on day 0 in males at all dose levels and at 100 and 500 mg/kg bw in females compared to control. Increased activity was observed at the low dose in both sexes (100 mg/kg) and decreased activity was observed at 500 and 1000 mg/kg bw in males and 500 mg/kg bw in females. No effect was observed at 250 mg/kg bw in females. Effects on activity recovered in surviving animals within seven days following treatment.

Table 6.7-3. Motor Activity (MA) (% difference from control)[a] – main study

	Males			Females		
Dose (ppm)	**100**	**500**	**1000**	**100**	**250**	**500**
Pre-treatment	+6	-8	+5	-4	+6	-7
Day 0	**+40***	**-50***	**-63***	**+54***	-14	**-51***
Day 7	-3	**-26***	+22	-9	+8	+4
Day 14	+7	-23	+19	-7	-7	-14

[a] Percent greater (=) or less (-) than concurrent control.
* Summary session motor activity was significantly different from control ($p \leq 0.05$; ANOVA) Differences from control that are considered biologically significant are shown in bold type.

In the supplemental study for the overall 90-minute test session, there were no statistically-significant or biologically significant differences in activity for males or females at the 20 or 50 mg/kg dose levels. The one instance in which motor activity exceeded 20 % from control involved a 24 % higher level of motor activity in high-dose males (Table 6.7-4). This is not considered biologically significant since 1) it was not statistically significant and 2) high-dose females were clearly not different from controls. Smaller apparent differences from control in high-dose females and in both sexes at the low dose are also considered incidental and not related to treatment with tebuconazole.

Table 6.7-4. Summary of Motor (MA) and Locomotor (LA) Activity Results (% difference from control)[a]

(suppl. study)

Dose (ppm)	Males				Females			
	20		50		20		50	
	MA	LA	MA	LA	MA	LA	MA	LA
Day 0	+7	+8	+24	+19	+7	+12	-3	+10

[a] Percent greater (=) or less (-) than concurrent control
Activity is not significantly different from control ($p < 0.05$; ANOVA)

Overall, effects on the figure-eight maze test were seen in males and females from 100 mg/kg bw, but not at 50 or 20 mg/kg bw. Based on these results, the NOAEL for measures of motor and locomotor activity is 50 mg/kg bw for males and females.

Functional Observational Battery (FOB) and Motor Activity Testing
The FOB test showed neurobehavioral effects on day 0 at 500 and 1000 mg/kg bw in males and at 100, 250, 500 mg/kg bw in females. Evidence of toxicity increased with dose, with minimal effects (increased activity in the open field – arousal) evident in females that received the 100 mg/kg dose and more severe toxicity evident at higher dose levels including: gait incoordination in the home cage and open field; decreased activity; increased activity in the open field (arousal), relative to controls; a higher incidence of animals standing in the home cage relative to controls; red lacrimation; diminished responses to approach, touch, auditory and tail pinch stimuli; impaired aerial righting; lower body temperature; and reduced hind limb grip strength performance. All signs of toxicity resolved in all dose groups in male and females by the next observation period on day 7. Overall, treatment-related effects on FOB tests were seen from 100 mg/kg bw in females.

In the supplemental study no compound-related effects on FOB tests were evident at the time of peak effect on the day of treatment (day 0) in males or females at either dose level.

Pathology
Animals that died during the post-treatment period showed evident gross lesions (nasal discharge and/or wetness and staining of the ventrum) in the highest dose males, and (bilateral red discoloration of the lungs) in the mid-dose males. No compound related gross lesions were observed in males or females that survived to terminal sacrifice, 15 days following treatment. There were no microscopic lesions at the highest dose males or females. Thus, tissues from animals that received a lower dose of tebuconazole were not examined.

Conclusion
This acute oral neurotoxicity study of tebuconazole was investigated in accordance with guideline and GLP. An overall dose-related increase in clinical signs of toxicity was observed from 500 mg/kg bw, with death occurring in males at the top dose of 1000 mg/kg bw. However, possible neurotoxic effects on FOB tests (females) and on the figure-eight maze test (males/females) were seen from the lowest dose tested of 100 mg/kg bw. Generalised and neurotoxic signs of toxicity were most pronounced a few hours after treatment, but all signs of toxicity were recovered 7 days after treatment. The results of this study showed evidence of acute neurotoxicity of tebuconazole in the Fisher 344 rat from a dose of 100 mg/kg bw.

Since an overall NOAEL was not originally established in this study, a follow-up study was conducted at 20 mg/kg bw and 50 mg/kg bw dose levels to establish a NOAEL for the endpoints (FOB and motor activity) that were affected from the lowest dose of 100 mg/kg bw. The results of the supplementary study at lower dose levels established 50 mg/kg bw as an overall NOAEL for both generalised toxicity and neurotoxicity in both sexes. These were the same NOAEL values agreed during the first review of tebuconazole.

2)

Previous evaluation:	None – submitted for purpose of renewal (study owned by EU Tebuconazole Task Force)

Study ID	B.6.7.1.1/02
Study title	Tebuconazole (technical grade), Acute oral neurotoxicity study in the rat
Test substance	tebuconazole
Purity (%) **Batch no.**	99.3 2008040703

Test animals	Rat, male and female, Wistar Han™:HsdRccHan™:WIST
Groups	10/sex/dose
Dose	0 (vehicle control), 100, 500 and 1500 mg/kg bw Administered once on Day 1
Route	oral (gavage)
Vehicle	Arachis oil BP
GLP	Yes
Guideline	OECD Guideline 424 (1997)
Deviation	The following deviations from the OECD-Guideline 424 (1997) occurred: None
Acceptable	Acceptable
NOAEL	Neurotoxicity: 500 mg/kg bw. Generalised toxicity: 100 mg/kg bw.
Effects at the LOAEL	Neurotoxicity: based on a range of signs indicative of neurotoxicity at the top dose (1500 mg/kg bw). Generalised toxicity: based on effects on body weights in females at the next highest dose (500 mg/kg bw).

Methods
Tebuconazole was administered orally by gavage to young Wistar rats, using doses of 0, 100, 500, 1500 mg/kg bw (males and females). The post-treatment period was 15 days. Neurobehavioral evaluation was conducted on all 10 rats/sex/dose level. Body weights were recorded on day 1, 4, 8, 11 and 15 (prior to terminal kill). Detailed examinations for clinical signs of toxicity were performed on a daily basis. Functional performance tests for motor activity and forelimb/hind limb grip strength were performed on days 1, 7 and 8. Five males and 5 females from each of the treatment and control groups were subject to whole body perfusion. Gross necropsies were performed on all other animals and histopathological examinations of neural tissue was performed on all perfused animals from the control and high dose groups.

Results
Mortality
There were no unscheduled deaths during the study.

Body weight
Body weight losses were evident following treatment for animals of either sex treated with 1500 mg/kg bw; however recovery was evident from day 4 onwards. Reduced body weight gains were also evident at 500 mg/kg bw and actual body weight losses were evident for females following treatment from 500 mg/kg bw. No effects occurred at 100 mg/kg bw.

Organ weight
There were no treatment-related changes in brain weight, both absolute and relative to terminal body weight.

Clinical observations
Clinically observable signs of potential neurotoxicty were evident five hours following treatment on day 1 for three males and four females treated with 1500 mg/kg bw. Signs included piloerection and hunched posture, decreased respiratory rate, piloerection and ataxia. A complete regression in clinical signs of toxicity was evident from day 2 onwards. No clinically observable signs of toxicity were evident for animals of either sex treated with 500 and 100 mg/kg bw.

Neurotoxicity
Open-field arena behavioural observations confirmed the clinical signs observed on day 1 at 1500 mg/kg bw. No such signs were evident on days 7 and 14. No treatment-related changes in behaviour were evident at 500 or 100 mg/kg bw.

No statistically significant treatment-related differences in sensory reactivity or grip strength were detected for treated animals, compared to controls.
No toxicologically significant differences in motor activity were evident for treated animals, compared to controls. A slight reduction in overall motor activity (overall mobile activity and final 20% of activity) was evident for males treated with 1500 mg/kg bw following dosing on day 1. Motor activity was also lower for these animals on days 7 and 14. However, there were no statistically significant intergroup differences. No such effects were seen

in females at the high dose (1500 mg/kg bw), or any animals at 100 or 500 mg/kg bw.

Pathology
No macroscopic abnormalities were noted and no treatment-related microscopic changes were detected in the neural tissues examined.

Conclusions
Single oral (gavage) administration of tebuconazole (technical grade) to Wistar rats at dose levels of 100, 500 and 1500 mg/kg bw resulted in treatment-related effects (effects on body weight and clinical signs of toxicity) at 1500 mg/kg bw in both sexes, with effects on body weights also seen in females treated with 500 mg/kg bw. Pilo-erection, hunched posture, ataxia and decreased respiratory rate were observed five hours after treatment during the clinical observations for animals of either sex treated with 1500 mg/kg bw. These signs were confirmed by behavioural assessments undertaken on day 1. Hypothermia was also recorded for one 1500 mg/kg bw female. Collectively, these signs may be indicative of neurotoxicity. Complete regression of these signs however was evident on the day after treatment. Remaining treatment-related differences were confined to body weight losses observed at 1500 mg/kg bw in both sexes and for females treated with 500 mg/kg bw. Neuropathological examinations did not reveal any treatment-related changes.

In conclusion, single oral (gavage) administration of tebuconazole (technical grade) to Wistar rats, at dose levels of 100, 500 and 1500 mg/kg bw, resulted in neurotoxic effects at 1500 mg/kg bw. The effects were fully reversible. On this basis, a NOAEL for acute neurotoxicity was established at 500 mg/kg bw. A NOAEL of 100 mg/kg bw was established for generalised toxicity based on effects on body weights in females.

B.6.7.1.2. ***Sub-chronic dietary neurotoxicity study in the rat***

Previous evaluation:	In Tebuconazole DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.7.1.2/01
Study title	A subchronic dietary neurotoxicity screening study with technical grade tebuconazole in Fischer 344 rats
Test substance	Tebuconazole
Purity (%) **Batch no.**	96.7 - 98.2 Batch no.: 603-0013
Test animals	Male and female Fischer 344 CDF (F-344) rats/BR from Sasco, Inc., Madison, WI
Groups	12 rats/sex/dietary level
Dose	0, 100, 400 and 1600 ppm for males and females Equivalent to (mg/kg bw/day): 0, 7, 57, 29.2 and 107 (males); 0, 8.81, 34.0 and 122 (females) For 13-weeks
Route	Oral, Dietary intake
Vehicle	Corn oil at 1% by weight of the diet, a small amount of acetone was used in the preparation of the diet.
GLP	Yes
Guideline	Similar to OECD Guideline 424
Deviation	The following deviations from the OECD-Guideline 424 (1997) occurred: None
Acceptable	Acceptable
NOAEL	Neurotoxicity: 1600 ppm, equivalent to 177 and 122 mg/kg bw/d in males and females respectively. Generalised toxicity: 400 ppm, equivalent to 29 and 34 mg/kg bw/d in males and females respectively.
Effects at the LOAEL	Neurotoxicity: No evidence of neurotoxicity was observed at the highest concentration (1600 ppm). Generalised toxicity: Based on decreased food consumption and body weight gain at the top dose (1600 ppm).

Methods

Tebuconazole was administered in the diet for 13 weeks to young-adult Fischer 344 rats, using nominal concentrations of 0, 100, 400 and 1600 ppm for males and females. Analytical chemistry included analysis of feed, corn oil and water, analysis of test substance and of test substance in the diet. Neurobehavioral evaluation was conducted on all 12 rats/sex/dietary level. Clinical observations, body weight and food consumption were performed on a weekly basis. Automated measurements of motor and locomotor activity (figure-eight maze) together with functional observational battery (FOB) were performed, together with ophthalmic examinations, brain weight determination and a gross necropsy. Skeletal muscle, peripheral nerves, eyes (with optic nerves) and tissues from the central nervous system were also examined microscopically for lesions.

Results
Average daily intake of active ingredient was 0, 7.57, 29.2, and 107 mg/kg/day (males) and 0, 8.81, 34.0, and 122 mg/kg/day (females).

Mortality
There were no deaths before the scheduled terminal sacrifice

Body weight
Body weight and food consumption were reduced in males and females at the 1600 ppm dietary level.

Table 6.7-5. Body weight data (mean (g) ± standard deviation and % change compared to control)

Males	**Nominal Dose (ppm)**			
Days	**0**	**100**	**400**	**1600**
0	169.0 ± 7.2	168.3 ± 10.9 *± 0 %*	166.0 ± 9.1 *- 2 %*	168.4 ± 7.6 *± 0 %*
7	198.7 ± 9.4	197.2 ± 12.2 *- 1 %*	189.9 ± 10.6 *- 4 %*	185.7* ± 10.9 *- 7 %*
14	228.6 ± 13.0	226.1 ± 14.7 *- 1 %*	217.4 ± 13.0 *- 5 %*	214.0* ± 11.9 *- 6 %*
21	237.5 ± 14.1	236.9 ± 15.3 *± 0 %*	228.8 ± 14.8 *- 4 %*	225.0 ± 14.1 *- 5 %*
28	253.3 ± 16.1	252.5 ± 18.4 *± 0 %*	241.5 ± 13.3 *- 5 %*	239.3 ± 12.8 *- 6 %*
35	269.6 ± 16.6	269.2 ± 19.3 *± 0 %*	258.2 ± 12.1 *- 4 %*	254.4 ± 13.0 *- 6 %*
42	284.6 ± 16.3	284.3 ± 21.2 *± 0 %*	273.0 ± 13.0 *- 4 %*	266.4* ± 13.8 *- 6 %*
49	291.4 ± 16.7	290.3 ± 23.6 *± 0 %*	279.5 ± 13.4 *- 4 %*	270.5* ± 14.4 *- 7 %*
56	300.6 ± 16.4	298.7 ± 22.7 *- 1 %*	289.3 ± 14.6 *- 4 %*	277.4* ± 16.3 *- 8 %*
63	311.3 ± 16.7	309.2 ± 22.5 *- 1 %*	299.8 ± 15.0 *- 4 %*	286.2* ± 16.2 *- 8 %*
70	320.9 ± 16.7	319.5 ± 24.3 *± 0 %*	307.8 ± 15.4 *- 4 %*	294.6* ± 16.7 *- 8 %*
77	329.2 ± 17.1	327.4 ± 25.5 *- 1 %*	318.9 ± 15.4 *- 3 %*	303.2* ± 16.9 *- 8 %*
84	332.0 ± 15.1	329.9 ± 26.2 *- 1 %*	321.5 ± 17.6 *- 3 %*	305.1* ± 18.0 *- 8 %*
91	341.1 ± 17.2	339.2 ± 28.8 *- 1 %*	329.4 ± 17.7 *- 3 %*	313.3* ± 17.2 *- 8 %*
BW gain d 0 - 91	172.1	170.9 *- 1 %*	163.4 *- 5 %*	144.9 *- 16 %*
Females	**Nominal Dose (ppm)**			
Days	**0**	**100**	**400**	**1600**
0	117.6 ± 5.6	119.3 ± 5.7 *+ 1 %*	118.7 ± 6.2 *+ 1 %*	119.4 ± 4.6 *+ 2 %*
7	130.6 ± 5.6	130.4 ± 6.0	128.2 ± 5.7	124.1* ± 5.4

Males	Nominal Dose (ppm)			
Days	**0**	**100**	**400**	**1600**
		± 0 %	*- 2 %*	*- 5 %*
14	145.0 ± 4.8	142.7 ± 7.6 *+ 5 %*	140.7 ± 5.9 *- 3 %*	135.5* ± 5.3 *- 7 %*
21	150.2 ± 4.8	150.5 ± 8.6 *± 0 %*	149.0 ± 7.1 *- 1 %*	141.0* ± 5.0 *- 6 %*
28	156.2 ± 4.2	157.1 ± 9.7 *+ 1 %*	156.7 ± 6.7 *± 0 %*	147.5* ± 7.3 *- 6 %*
35	164.8 ± 5.2	165.4 ± 9.3 *± 0 %*	164.4 ± 6.8 *± 0 %*	154.1* ± 7.5 *- 6 %*
42	170.5 ± 4.9	168.6 ± 10.8 *- 1 %*	169.5 ± 7.5 *- 1 %*	159.1* ± 6.5 *- 7 %*
49	174.1 ± 6.3	174.7 ± 11.2 *± 0 %*	174.5 ± 8.3 *± 0 %*	162.5* ± 6.9 *- 7 %*
56	176.7 ± 5.7	175.2 ± 10.5 *- 1 %*	176.2 ± 8.0 *± 0 %*	165.2* ± 7.4 *- 7 %*
63	180.8 ± 4.8	180.8 ± 10.5 *± 0 %*	180.6 ± 7.5 *± 0 %*	169.5* ± 7.9 *- 6 %*
70	184.8 ± 6.1	184.1 ± 11.4 *± 0 %*	185.0 ± 7.5 *± 0 %*	172.8* ± 8.7 *- 6 %*
77	187.9 ± 5.3	186.2 ± 12.2 *- 1 %*	188.1 ± 8.5 *± 0 %*	174.0* ± 8.2 *- 7 %*
84	190.6 ± 6.2	187.8 ± 12.2 *- 1 %*	189.1 ± 9.1 *- 1 %*	177.0* ± 9.6 *- 7 %*
91	192.7 ± 6.5	192.0 ± 12.7 *± 0 %*	191.1 ± 8.7 *- 1 %*	179.7* ± 10.4 *- 7 %*
BW gain d 0 – 91	75.1	72.7 *- 3 %*	72.4 *- 4 %*	60.3 *- 20 %*

BW: Body weight
* Significantly different from control at p≤ 0.05

Organ weight
There was no significant difference in brain weight at any dietary exposure level. The increase in relative brain weight for high-dose males and females is attributed to the combination of comparable brain weight and lower body weight for those groups, relative to controls, as can be seen in the following table.

Table 6.7-6. Terminal body weights and brain weights (absolute and relative)

Parameter	Dose (ppm)			
	0	**100**	**400**	**1600**
Males				
Number	6	6	6	6
Terminal body weight (g)	337.2 ± 16.7	346.4 ± 22.6	339.3 ± 19.9	308.3 ± 14.8
Brain weight (g)	1.758 ± 0.064	1.774 ± 0.127	1.791 ± 0.098	1.763 ± 0.056
Brain/body weight (%)	0.523 ± 0.038	0.513 ± 0.034	0.529 ± 0.034	0.573 ± 0.022
Females				
Number	6	6	6	6
Terminal body weight (g)	194.1 ± 7.4	195.9 ± 16.7	195.9 ± 11.9	187.4 ± 12.4
Brain weight (g)	1.736 ± 0.054	1.694 ± 0.083	1.792 ± 0.089	1.757 ± 0.102
Brain/body weight (%)	0.895 ± 0.042	0.870 ± 0.081	0.916 ± 0.030	0.941 ± 0.085

Clinical signs
There were no compound-related clinical signs in males or females.

Functional Observational Battery (FOB) and Motor Activity Testing
There were no compound-related findings in the functional observational battery (FOB) or in the automated measures of motor and locomotor activity. Compound-related effects on interval motor and locomotor activity

were not evident in males or females at any dietary level. Various minimal differences in activity for both sexes in various dietary groups (both increases and decreases) are considered to be incidental and not related to treatment. Likewise, habituation was not affected by exposure to tebuconazole at any dietary level.

Table 6.7-7. Motor (MA) and Locomotor (LA) Activity (percent difference from control)

Dose (ppm)	Males						Females					
	100		400		1600		100		400		1600	
	MA	LA	MA	LA	MA	LA	MA	LA	MA	LA	MA	LA
Pre-treatment	+ 9	+ 2	+ 13	+ 9	+ 3	- 6	+ 3	+ 10	+ 6	+ 9	- 9	- 7
Week 4	+ 4	+ 13	- 14	- 9	- 7	- 2	+ 27	+ 30	+ 11	+ 16	- 21	- 24
Week 8	- 3	- 9	+ 9	+ 1	+ 19	+ 3	+ 5	+ 8	- 4	- 1	- 23	- 24
Week 13	- 14	- 11	+ 9	+ 7	+ 12	+ 9	+ 24	+ 30	+ 18	+ 14	+ 1	- 2

Ophthalmology
There were no compound-related ophthalmic findings.

Pathology
There were no compound-related microscopic lesions in neural tissues or skeletal muscles. At necropsy no compound related lesions were observed.

Conclusion
This sub-chronic study (13-week) of dietary administration of tebuconazole was in accordance with an EPA guideline and GLP. No evidence of neurotoxicity was observed at the highest concentration (1600 ppm). Therefore a NOAEL for repeated dose neurotoxicity of 1600 ppm (177/122 mg/kg bw/d in M/F) was established. Based on decreased food consumption and body weight gain the maximum tolerated dose (MTD) was 1600 ppm and the NOAEL for generalised toxicity was 400 ppm corresponding to 29 mg/kg bw/day in males and 34 mg/kg bw/day in females. All effects of treatment are considered reversible, with complete recovery expected with discontinuation of exposure. These were the same NOAEL values agreed during the first review of tebuconazole.

B.6.7.2. Delayed polyneuropathy studies

No delayed polyneuropathy studies have been submitted by either task force. According to the new data requirements (Commission Regulation (EU) No 283/2013), delayed polyneuropathy studies shall be performed for active substances of similar or related structures to those capable of inducing delayed polyneuropathy such as organophosphorus compounds. In the case of tebuconazole, no signs of delayed neurotoxicity were seen in subacute, subchronic or chronic studies and although there is some evidence of acute neurotoxicity, no neurotoxicity was seen after repeated exposure for 90 days. Therefore, this type of study is not necessary.

B.6.7.3. Literature data

One publication of potential relevance to neurotoxicity has been identified.

Previous evaluation	None – publication submitted for the purpose of renewal

Study ID	B.6.7.1.3/01
Author(s)	Heusinkveld *et al.*, (2013)
Study title and journal	Azole Fungicides Disturb Intracellular Ca^{2+} in an Additive Manner in Dopaminergic PC12 Cells. Toxicological Sciences, 134(2), 374 - 381.
Test substance	Tebuconazole (and other azole fungicides)
Purity (%) **Batch no.**	Not specified Not specified
GLP	No
Guideline	No
Deviation	n/a
Reliability	Reliable with restrictions

Relevance to hazard assessment	Limited relevance as paper describes *in vitro* investigations only.

Methods
Rat dopaminergic pheochromocytoma cells (PC12 cells) were treated *in vitro* with 100 µM tebuconazole for 24 hours. Following treatment, the production of ROS and changes in the levels of intracellular Ca2+ were investigated.

Results and conclusions
Tebuconazole did not increase ROS production. However, tebuconazole induced a non-specific inhibition of voltage-gated calcium channels (VGCCs) and associated depolarization-evoked calcium influx.

The UK RMS deems that, given the availability of *in vivo* neurotoxicity studies, these *in vitro* findings are of limited relevance to the hazard assessment of tebuconazole.

(Heusinkveld *et al.*, 2013)

B.6.7.4. Summary of Neurotoxicity

The neurotoxic potential of tebuconazole has been investigated in rats in two oral (gavage) acute neurotoxicity studies and in a 90-day dietary neurotoxicity study. There is also an *in vitro* investigation from the open literature which is of limited relevance.

The following key conclusions were obtained from the evaluation of the neurotoxicity information:

- An overall acute neurotoxicity NOAEL of 50 mg/kg bw could be established. No signs of neurotoxicity (acute oral rat.
- Tebuconazole was not neurotoxic at the highest-tested dose of 122 mg/kg bw/d (90-day rat). General toxicity was seen (NOAEL 29 mg/kg bw/d) in this repeat-dose neurotoxicity study.

Study **Doses tested**	**Sex**	**NO(A)EL**	**LO(A)EL** **mg/kg bw/d (ppm)**	**Main findings at LO(A)EL**	**Reference**
Acute neurotoxicity, Oral/gavage, Rat/ Fischer 344 CDF(F-344) Males: 0, 100, 500, 1000 mg/kg bw; Females: 0, 100, 250, 500 mg/kg bw Supplementary study: 20 and 50 mg/kg bw (M/F) (Bayer TF)	M/F	*Generalised toxicity and acute neurotoxicity* 50	*Generalised toxicity and acute neurotoxicity* 100	*Neurotoxicity* Increased activity in the FOB (F only) and in the figure-eight maze (both sexes) *Generalised toxicity* Clinical signs of toxicity	B.6.7.1.1/01
Acute neurotoxicity, Oral/gavage, Rat/ Wistar HanTM:HsdRccHanTM:WIST 0, 100, 500, 1500 mg/kg (M/F) (EU Tebuconazole Task Force)	M/F	*Acute neurotoxicity* *500*	*Acute neurotoxicity* *1500*	*Neurotoxicity* Pilo-erection, hunched posture, ataxia and decreased respiratory rate confirmed by open-field arena behavioural observations in both sexes treated with 1500 mg/kg bw	B.6.7.1.1/02

Study Doses tested	Sex	NO(A)EL	LO(A)EL mg/kg bw/d (ppm)	Main findings at LO(A)EL	Reference
		Generalised toxicity *100*	*Generalised toxicity* *500*	*Generalised toxicity* Body weight losses observed at 1500 mg/kg bw in both sexes and for females treated with 500 mg/kg bw	
Sub-chronic neurotoxicity, Oral/diet, Rat/ Fischer 344 CDF(F-344) 0, 100, 400, 1600 ppm (M/F: 0/0, 7.57/8.81, 29.2/34.0, 107/122 mg/kg bw/d (Bayer TF)	M/F	*Neurotoxicity* 177/122 (1600 ppm) *Generalised toxicity* 29/34 (400 ppm)	*Neurotoxicity* >177/122 (>1600 ppm) *Generalised toxicity* 177/122 (1600 ppm)	*Neurotoxicity* No neurotoxicity observed. *Generalised toxicity* Significantly reduced body weight (up to -8 %/-7% in M/F), reduced overall body weight gain (-16 %/-20 % in M/F) and significantly reduced food consumption (up to -9 %/-14 % in M/F)	B.6.7.1.2/01

Acute neurotoxicity (increased activity during the FOB observations and effects on performance in the maze test) was observed from 100 mg/kg bw in Fisher 344 rats in association with generalised toxicity (clinical signs of toxicity). A (generalised toxicity and acute neurotoxicity) NOAEL of 50 mg/kg bw was identified from this study. In a second study in Wistar rats, acute neurotoxicity (ataxia) was seen at a much higher dose of 1500 mg/kg bw, with generalised toxicity (body weight loss) occurring from 500 mg/kg bw. It is possible that the different responses observed in the two studies could be due to the different strains of rats used. An overall acute neurotoxicity NOAEL of 50 mg/kg bw could be established.

No neurotoxicity was seen in the 90-day study up to the top dose of 177/122 mg/kg bw/d at which generalised toxicity (decreased body weight, body weight gain and food consumption) occurred.

B.6.8. OTHER TOXICOLOGICAL STUDIES

B.6.8.1. Toxicity studies on metabolites and relevant impurities

No toxicological information was originally provided by either the Bayer TF or the EU Tebuconazole Task Force on a range of metabolites identified in the residue evaluation (reference Volume 3 CA B 7). However, for the four triazole-derived metabolites (1,2,4-triazole, triazole alanine, triazole lactic acid, and triazole acetic acid) reference values have already been established in an EFSA Conclusion (2018). If a risk assessment of these triazole-derived metabolites were to be required, the following reference values should be applied (see below).

The Bayer and EU Tebuconazole Task Force have recently submitted on request by the RMS a report to address the other metabolites (beyond the TDM) identified in the residue evaluation. However, these were submitted far too late to be taken into account in this version of the RAR. For metabolites M03 and M06, toxicological information was already considered during the first review. The RMS notes that additional information submitted late in the process includes (Q)SAR data; however the data requirements of Regulation 283/2013 may not have been met based on the potential lack of reliable information/data on reproductive toxicity and carcinogenicity of metabolites.

M03 - tebuconazole-1-hydroxy
M03 was primarily excreted in the rat faeces with about 30% (see Volume 3 B6 Table 6.1-15) in studies submitted in the original DAR (B.6.1.1.3/01, using phenyl-UL-^{14}C labelled tebuconazole) while the amount in the urine was up to 0.1 / 0.3 % (m/f) of the administered dose after administration at 2 mg/kg bw phenyl labelled tebuconazole and with up to 2.2 / 0.3 (m/f) of the administered dose after administration at 20 mg/kg bw of triazole labelled tebuconazole. The amount in urine is still below 10%, even when adding the amount in urine from the **M11** (tebuconazole-1-OH-glucuronide) which is a conjugate of M03. **Thus, M03 cannot be considered as covered by the parent substance.**

M06 - tebuconazole-carboxylic acid
M06 is a major rat metabolite in female rats but not in males (see Volume 3 B6 Table 6.1-14). In studies submitted in the original DAR (B.6.1.1.3/01, using phenyl-UL-^{14}C labelled tebuconazole) M06 was found in the urine in rats with up to 1.8 / 12.5 % (m/f) of the administered dose after 2 mg/kg bw phenyl labelled tebuconazole and with up to 1.6 / 9.7 % (m/f) of the absorbed radioactivity after 20 mg/kg bw of triazole labelled tebuconazole. Since the amount in urine from males are substantial lower than 10% **M06 cannot be considered as covered by the parent substance.**

Triazole Derived Metabolites - TDM
Potential soil, groundwater, plant and livestock metabolites of tebuconazole include 1,2,4-triazole, triazole alanine, triazole lactic acid, and triazole acetic acid (triazole derived metabolites – TDM). Toxicological data have been submitted and collated on these metabolites, common to triazole fungicidal compounds, and their toxicological evaluation, including setting of reference values has been considered under a separate EU process. For each of these metabolites, the following reference values were agreed at EU level (EFSA Conclusion, June 2018).

Table 6.8-1. Summary of agreed reference values for the four triazole metabolites (EFSA Conclusion, 2018)

Metabolite	Ref. values (derived at the Pesticides Peer Review TC 162 (Sept 2017)	Study	Effect observed at the LOAEL	UF	Previously set Ref. values (derived at the PRAPeR 14, Jan 2007)	Previously set UF
1,2,4-triazole: ADI	**0.023 mg/kg bw per day**	Newly submitted rat 12-month study	Decreased body weight gain	300	0.02 mg/kg bw per day (rat multigeneration study)	1000
ARfD	**0.1 mg/kg**	Rabbit	Decreased body	300	0.06 mg/kg bw	500

	bw	developmental study	weight gain		(rat developmental study)	
Triazole Alanine: ADI	**0.3 mg/kg bw per day**	Newly submitted rabbit developmental study	Increased incidence of hyoid angulated alae	100	0.1 mg/kg bw per day (rat developmental study)	1000
ARfD	**0.3 mg/kg bw**	Newly submitted rabbit developmental study	Increased incidence of hyoid angulated alae	100	0.1 mg/kg bw (rat developmental study)	1000
Triazole Acetic Acid: ADI	**1 mg/kg bw per day**	Newly submitted rat 2-generation and rabbit developmental studies	Maternal and developmental toxicity	100	0.02 mg/kg bw per day (derived from 1,2,4-T)	1000
ARfD	**1 mg/kg bw**	Newly submitted rat 2-generation and rabbit developmental studies	Maternal and developmental toxicity	100	0.06 mg/kg bw (derived from 1,2,4-T)	1000
Triazole Lactic Acid: ADI	**0.3 mg/kg bw per day**	Bridging from TA			Not set	
ARfD	**0.3 mg/kg bw**				Not set	

B.6.8.2. Supplementary studies on the active substance

B.6.8.2.1. *Combination toxicity*

The combination toxicity of tebuconazole with triadimenol or dichlofluanid was investigated in two acute studies conducted via the oral route. The available studies, owned by Bayer Task Force (TF), were evaluated in the original DAR (2006), peer reviewed at EU level (EFSA Conclusion, 2008), and were considered acceptable.

1)

Previous evaluation:	In Tebuconazole DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.8.2.1/01
Study title	HWG 1608 and KWG 0519 / HWG 1608 and KUE 13032c - Combination toxicity study
Test substance	Tebuconazole + dichlofluanid in Cremophor EL/demineralized water
Purity (%) **Batch no.**	94.7 Batch no. 16002/86

Test animals	Male Wistar rats/Bor:WISW (SPF-Cpb)
Groups	5 male rats
Dose	5000 mg/kg bw (tebuconazole) (dichlofluanid) (tebuconazole + dichlofluanid)
Route	Oral by gavage (fasted animals)
Vehicle	Cremophor EL/demineralized water (2 %)
GLP	No, at the time the study was performed GLP was not compulsory.
Guideline	OECD Guideline 401
Deviation	The following deviations from the OECD-Guideline 401 (1987) occurred: - None
Acceptable	Acceptable – supplementary
LD50	> 5000 mg/kg bw.

Methods
Tebuconazole and dichlofluanid, formulated in Cremophor EL/demineralized water (2 %), were administered oral by gavage to 5 male rats in a single dose at dose 5000 mg/kg body weight. The post-treatment observation period lasted 14 days. Clinical signs were recorded at the day of administration and further once a day. Body weight was recorded at the day of administration and further on a weekly basis. Necropsy was performed at the end of the post-treatment period, and all animals were subjected to a gross pathology examination. Animals that died under the observation period were also subjected to a gross pathology examination.

Results
Mortality
One rat died at day six.

Body weight
There were no treatment related effects on body weight and body weight development at the end of the study.

Clinical signs
Bristled fur, apathy, reduced motility, spastic gait, staggering, dyspnoea, salivation, diarrhoea was observed.

Pathology
At termination abnormal findings in the liver (thin yellowish layer), lungs (patchy, dark spots) and scar like changes were observed.

Conclusion
This acute oral toxicity study on a formulation containing tebuconazole and dichlofluanid was performed in accordance with OECD/EU guidelines. The test formulation showed no significant oral toxicity in rats. The following LD_{50} value was established: LD_{50}: > 5000 mg/kg bw.

2)

Previous evaluation:	In Tebuconazole DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.8.2.1/02
Study title	HWG 1608 and KWG 0519 (c.n. : Triadimenol)/ HWG 1608 and KUE 13032c (c.n. : Dichlofluanid) - Combination toxicity study
Test substance	Tebuconazole and triadimenol
Purity (%) **Batch no.**	Tebuconazole (94.7); Triadimenol (94.7) Tebuconazole (16002/86); Triadimenol (203519028)
Test animals	Male Wistar rats/Bor:WISW (SPF-Cpb)
Groups	5 male rats/dose
Dose	Tebuconazole: 5000 mg/kg bw Triadimenol: 710, 1000, 1600 mg/kg bw Tebuconazole + Triadimenol: 2000, 2240, 3000 mg/kg bw (equitoxic doses)
Route	Oral by gavage (fasted male rats)

Vehicle	Cremophor EL/demineralized water (2 %)
GLP	No, at the time the study was performed GLP was not compulsory.
Guideline	OECD Guideline 401
Deviation	The following deviations from the OECD-Guideline 401 (1987) occurred: - None
Acceptable	Acceptable – supplementary
LD50	3046 mg/kg bw (calculated); 2424 mg/kg bw (experimental).

Methods

Tebuconazole and triadimenol, formulated in Cremophor EL/demineralised water (2%), was administered oral by gavage in a single dose to 5 male rats in each dosing group. In the combination study equitoxic doses were used corresponding to tebuconazole (82.12%) + triadimenol (17.88%). The post-treatment observation period lasted 14 days. Clinical signs were recorded at the day of administration and further once a day. Body weight was recorded at the day of administration and further on a weekly basis. Necropsy was performed at the end of the post-treatment period, and all animals were subjected to a gross pathology examination. Animals that died under the observation period were also subjected to a gross pathology examination.

Results

Mortality

Tebuconazole (a total of 1 rat), triadimenol (a total of 7 rats from 710 to 1600 mg/kg bw), tebuconazole + triadimenol (a total of 7 rats from 200 to 3000 mg/kg bw).
(Table 6.8-14)

Body weight

Body weight loss was observed but it was reversible until the end of post-treatment observation period.

Clinical signs

Bristled fur, pallor, apathy, reduced and later increased motility, spastic gait, staggering, cramps, partly convulsions, lateral recumbency, salivation, lacrimation, dyspnoea was observed.

Pathology

Changes were observed in the lung (distended, dark-patchy), the liver (pale, lobulation), the spleen (pale), the kidney renal pelvis (pale, structure indistinct, reddened) and the glandular stomach mucosa (reddened, ulcer-like foci). No treatment-related macroscopic changes were observed in animals sacrificed at termination except changes in the liver (partly hardened areas) and stomach (hardened areas in one animal).

Table 6.8-2. **Table for combination toxicity study**

Dose [mg/kg bw]	**Toxicological results***	**Duration of clinical signs**	**Time of death**
Tebuconazole			
5000	1/5/5	2h-12d	6d
LD_{50}: > 5000 mg/kg bw			
Triadimenol,			
710	1/5/5	30m-5d	3h
1000	2/5/5	20m-7d	1d-3d
1600	4/5/5	40m-6d	5h-2d
LD_{50}: 1089 mg/kg bw			
Tebuconazole (82.12%) + Triadimenol (17.88%)			
200	1/5/5	50m-8d	4d
2240	2/5/5	1h-8d	1d-6d
3000	4/5/5	40m-14d	1h-5d
LD_{50}: 3046 mg/kg bw (calculated); 2424 mg/kg bw (experimental)			

* First number = number of dead animals
Second number = number of animals with toxic signs
Third number = number of animals used

Conclusion
This acute combination oral toxicity study of tebuconazole and triadimenol was performed in accordance with OECD/EU guidelines. Equitoxic doses of tebuconazole and triadimenol administered orally to fasted rats resulted in a slightly potentiating effect. The following LD_{50} value was established: LD_{50}: 3046 mg/kg bw (calculated); 2424 mg/kg bw (experimental).

Summary of acute combination toxicity studies

The combination toxicity of tebuconazole with triadimenol or dichlofluanid was investigated in two acute studies conducted via the oral route. The available studies, owned by Bayer Task Force (TF), were evaluated in the original DAR (2006), peer reviewed at EU level (EFSA Conclusion, 2008), and were considered acceptable.

Type of study	Substance	Dose levels mg/kg bw/day	NOAEL males/ females mg/kg bw/day	LOAEL males/ females mg/kg bw/day	Findings	Reference
Acute combination study in rats Oral/gavage	Tebuconazole and triadimenol	M: 5000 (tebuconazole) 710 1000, 1600 (triadimenol) 2000, 2240, 3000 (tebuconazole + triadimenol)	LD_{50}: 3046 (calculated) 2424 (experimental).	-	Mortality: tebuconazole + triadimenol (1/5 at 200, 2/5 at 2240, and 5/5 at 3000 mg/kg bw, Tebuconazole (1/5 at 5000 mg/kg bw)	B.6.8.2.1/01
Acute combination study in rats Oral/gavage	Tebuconazole and dichlofluanid	M: 5000 mg/kg bw (tebuconazole) (dichlofluanid) (tebuconazole + dichlo-fluanid)	LD_{50}: > 5000 (calculated) 5000 (experiment-tal).	-	One rat died at day six.	B.6.2.1.1/02

The acute oral toxicity study on a formulation containing tebuconazole and dichlofluanid showed no significant oral toxicity in rats (LD_{50}: > 5000 mg/kg bw). It is noted that the presence of dichlofluanid does not lead to an increase in the acute oral toxicity of this combination compared to tebuconazole alone (oral LD50 > 5000 mg/kg bw). The acute combination oral toxicity of tebuconazole and triadimenol showed increased oral toxicity compared to administration of tebuconazole alone (LD50: 2424 mg/kg bw in combination vs >5000 mg/kg bw tebuconazole only).

B.6.8.2.2. *Acute intraperitoneal toxicity*

An acute intraperitoneal toxicity study, owned by Bayer TF, was evaluated in the original DAR (2006), peer reviewed at EU level (EFSA Conclusion, 2008), and considered acceptable.

Previous evaluation:	In Tebuconazole DAR (2006) for original approval (study owned by Bayer Task force)

Study ID	B.6.8.2.2/01
Study title	HWG 1608 - Study for acute toxicity
Test substance	(HWG 1608) Tebuconazole
Purity (%) **Batch no.**	97.1 Batch no. 16001/83
Test animals	Wistar rats/Bor:WISW (SPF-Cpb)
Groups	5-10 rats/sex/group. The animals were acclimatized for at least 5 days before treatment
Dose	Male: 50, 100, 500, 630, 710, 800, 900, 1000 mg/kg bw. (dosing volume 10 mL/kg bw) Female: 50, 100, 355, 400, 450, 560 mg/kg bw. (dosing volume 10 mL/kg bw)

Route	Intraperitoneal
Vehicle	Cremophor EL/water
GLP	No; at the time the study was performed GLP was not compulsory.
Guideline	None
Deviation	n/a
Acceptable	Acceptable – supplementary
LD50	LD50: 751 mg/kg bw (males) and LD50: 395 mg/kg bw (females).

Methods
The acute intraperitoneal toxicity of tebuconazole was investigated in 5-10 rats/sex/group. Tebuconazole was injected once into the abdominal cavities of male rats at 50, 100, 500, 630, 710, 800, 900, 1000 mg/kg bw (dosing volume 10 mL/kg bw) and of female rats at 50, 100, 355, 400, 450, 560 mg/kg bw. (dosing volume 10 mL/kg bw). The post-treatment observation period lasted 14 days. Clinical signs were recorded at the day of administration and further once a day. Body weight was recorded at the day of administration and further on a weekly basis. Necropsy was performed at the end of the post-treatment period, and all animals were subjected to a gross pathology examination. Animals that died under the observation period were also subjected to a gross pathology examination.

Results
Mortality
18 of 45 male rats (from 630-1000 mg/kg bw), 22 of 40 female rats (from 355-560 mg/kg bw) (table 6.2-5)

Clinical signs
Behavioural, breathing and motility disturbances, staggering, spastic gait, uncoordinated movements, poor reflexes, narcosis, convulsions, lateral or sternal recumbency were observed.

Pathology
During the observation period changes were observed in the lung (spotted to dark-red, distended), kidney (patchy, pale), spleen (patchy, pale), liver (patchy, swollen, liver lobes adherent to each other and to pancreas, diaphragm, stomach and fatty tissues), glandular stomach (reddened), walls of stomach thin and without structure, clear liquid in the abdominal cavity and whitish deposits on all abdominal organs. At termination changes were observed in the liver (swollen, adhesion) and spleen (covered with white skin).

Table 6.8-3. Acute intraperitoneal toxicity

Dose [mg/kg bw]	Toxicological results*	Duration of clinical signs	Time of death
Males			
50	0/0/5	-	-
100	0/5/5	50’ – 1d	-
500	0/5/5	16’ – 3d	-
630	1/5/5	8’ – 6d	1d
710	2/5/5	7’ – 6d	1d – 3d
800	6/10/10	14’ – 10d	1d – 3d
900	4/5/5	17’ – 3d	3h – 2d
1000	5/5/5	7’ – 1d	2h – 1d
LD_{50}: 751 mg/kg bw			
Females			
50	0/0/5	-	--
100	0/5/5	44’ – 1d	-
355	1/5/5	18’ – 6d	3d
400	3/5/5	13’ – 3d	1d-3d
450	8/10/10	10’ – 6d	1d-3d
560	10/10/10	9’ – 4d	1d-4d

LD_{50}: 395 mg/kg bw

* First number = number of dead animals
Second number = number of animals with toxic signs
Third number = number of animals used

Conclusion
Tebuconazole was slightly toxic to rats after acute intraperitoneal administration. The following LD_{50} values were established: LD_{50}: 751 mg/kg bw (males) and LD_{50}: 395 mg/kg bw (females).

B.6.8.2.3. ***Immunotoxicological potential***

No studies investigating the immunotoxic potential of tebuconazole were evaluated in the original DAR (2006). The immunotoxicity of tebuconazole has been investigated in a 28 day oral study submitted for the purpose of renewal by Bayer TF

Previous evaluation:	None – submitted for the purpose of renewal (study owned by Bayer Task Force)

Study ID	B.6.8.2.3/01
Study title	Tebuconazole: 28-day immunotoxicity study in the female Wistar rat by dietary administration
Test substance	Tebuconazole
Purity (%) Batch no.	97.5 Batch no: K689052
Test animals	Female wistar rats: Rj:WI (IOPS HAN)
Groups	10 animals/group
Dose	0, 100, 300, 1000 ppm (dose 8.1, 24.3, and 78.4 mg/kg respectively) Positive control: 3.5 mg/kg bw/day cyclophosphamide per gavage at 5 mL/kg bw
Route	Oral by diet
Vehicle	None
GLP	Yes
Guideline	US-EPA OPPTS 870.7800 (1998)
Deviation	None
Acceptable	Acceptable
NOAEL	No evidence of immunotoxic potential in female Wistar rats administered Tebuconazole continuously in the diet at levels up to 1000 ppm for at least 28 days. NOAEL for immunotoxicity was determined to be 1000 ppm in the diet corresponding to 78.4 mg/kg/day based on body weight.

Methods
Tebuconazole was administered to female wistar rats (10/group) via the diet at 0, 100, 300 and 1000 ppm (equivalent to 8.0, 24.3, and 78.4 mg/kg) for at least 28 days. An additional group received an immunosuppressive agent, Cyclophosphamide, daily by gavage for 28 days as a positive control. Mortality and clinical signs were recorded daily, and body weights were recorded weekly alongside food consumption. Detailed physical examinations were performed at least weekly during the treatment period. On day 26, four days prior to necroscopy, all animals were immunised with Sheep Red Blood Cell (SRBC) antigen by intravenous injection. Blood samples were taken on day 30 for SRBC-specific immunoglobulin M analysis. All animals were necroscopied and the spleen and thymus were weighed.

Results
Mortality
There were no mortalities during the study.

Clinical signs
There were no treatment-related clinical signs during the course of the study.

Body weight & Food consumption
At 1000 ppm, from day 8 to the end of the study, the mean body weight was 6-7% lower (not statistically significant) than the control group. At the end of the study, the overall mean body weight gain at the high dose was approximately 27% lower ($p \leq 0.01$) than the control group. At 300 and 100 ppm, body weight and body weight gain were unaffected by treatment with the test item tebuconazole. For animals treated with cyclophosphamide, there was no change in mean terminal body weight in treated animals when compared to controls

At 1000 ppm, mean food consumption was approximately 13% lower ($p \leq 0.05$) when compared to the control group, at the end of the study. At 300 and 100 ppm, mean food consumption was unaffected by treatment with the test item tebuconazole. Overall, there were effects on body weights and food consumption at 1000 ppm.
Immune response (SRBC-specific IgM response)
No treatment-related change was noted in anti-SRBC IgM concentrations up to 1000 ppm.

The high mean anti-SRBC IgM concentration observed in the control group confirmed the sensitisation of the animals. At 3.5 mg/kg/day cyclophosphamide, mean anti-SRBC IgM concentration was markedly lower (-85%, $p \leq 0.01$) when compared to the controls: this variation corresponds to the range usually observed with cyclophosphamide within laboratory conditions. A high inter-individual variability was noted in all the groups as usually observed with SRBC sensitisation.

Table 6.8-4.: SRBC-specific IgM (U/mL) mean ± standard deviation (% change when compared to controls) at Study day 30.

	Tebuconazole (ppm)				**Cyclophosphamide (mg/kg bw)**
	0	**100**	**300**	**1000**	**3.5**
SRBC-specific IgM	15854 ± 12669	13943 ± 18217 (-12%)	18673 ± 19777 (+18%)	14145 ± 5524 (-11%)	2412* ± 2200 (-85%)

*: $p \leq 0.01$

Organ weights
At 1000 ppm, mean spleen to body weight ratio was statistically significantly higher in females when compared to the controls. This change was considered to be treatment-related and linked to findings on red blood cells and the spleen observed in short-term studies (low erythrocytes counts and increased hemosiderin content in the spleen). This effect is therefore not considered to be a specific immunotoxic effect.

For animals treated with cyclophosphamide, mean absolute and relative spleen weights were statistically significantly lower when compared to the controls.

Table 6.8-5.:: Body and organ weights. Mean weight ±SD at scheduled sacrifice (% change when compared to controls)

	Tebuconazole (ppm)				**Cyclophosphamide (mg/kg bw)**
	0	**100**	**300**	**1000**	**3.5**
Body weight (g)	249.1 ±15.5	245.6 ±20.9 (-1%)	255.2 ±24.4 (+2%)	234.2 ±17.5 (-6%)	249.3 ±19.9 (0%)
Spleen (g)	0.724 ±0.071	0.767 ±0.172 (+6%)	0.795 ±0.157 (+10%)	0.928 ±0.198 (+28%)	0.627* ±0.090 (-13%)
Spleen / bw (%)	0.2915 ±0.0331	0.3099 ±0.0507 (+6%)	0.3112 ±0.0494 (+7%)	0.3952** ±0.0716 (+36%)	0.2530* ±0.0418 (-13%)
Thymus (g)	0.531 ±0.096	0.531 ±0.119 (0%)	0.627 ±0.143 (+18%)	0.557 ±0.104 (+5%)	0.448 ±0.109 (-16%)

Thymus / bw (%)	0.2143 ±0.0430	0.2160 ±0.0424 (+1%)	0.2451 ±0.0480 (+14%)	0.2372 ±0.0345 (+11%)	0.1807 ±0.0466 (-16%)

*: $p \leq 0.01$

Pathology
All the macroscopic changes were considered as incidental and not treatment-related.

Conclusion
This study revealed no evidence of immunotoxic potential in female Wistar rats administered tebuconazole continuously in the diet at levels up to 1000 ppm (78 mg/kg bw/d) for at least 28 days. This dose level produced significant systemic toxicity including decreased terminal body weight and body weight gain and increased spleen weight. Thus, the NOAEL for immunotoxicity was determined to be 1000 ppm in the diet corresponding to 78.4 mg/kg bw/d based on body weight. A NOAEL of 300 ppm (24 mg/kg bw/d) was identified for generalized/systemic toxicity.

Summary of immunotoxicity

The immunotoxicity potential of tebuconazole has been investigated in a specific immunotoxicity 28 day dietary study in rats. There was no evidence of immunotoxicity up to the top dose of 1000 ppm (78 mg/kg bw/d) at which significant generalised toxicity occurred.

B.6.8.3. Studies on endocrine disruption

An assessment of the human health endocrine disruption (ED) potential of the active substance tebuconazole in line with the new EFSA/ECHA guidance (https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2018.5311) for identification of endocrine disruptors (2018) was initially not performed/submitted as the application for renewal was submitted in 2017. In November 2018 the Bayer TF submitted an assessment where they concluded that tebuconazole is not an ED in relation to human health and in February 2019, the EU Tebuconazole Task Force submitted a position paper discussing the knowledge gaps and uncertainties surrounding the endocrine disrupting potential of tebuconazole. The UK-RMS did not evaluate these papers as they were submitted late in the process. However, UK-RMS evaluated the available data submitted (prior to Nov 2018) by the applicants and performed an ED assessment in line with the scientific criteria (Reg 605/2018). The UK-RMS cconsidered all the data informing on adverse effects potentially related to ED, information on endocrine activity, the link between the endocrine activity and the adverse effects and the specificity and human relevance of the effects. The UK-RMS assessment is kept in this RAR for transparency eventhough it was performed before the required use of the EFSA/ECHA ED GD (2018).

Due to Brexit the RAR was handed over to the co-RMS DK before the submission to EFSA. EFSA required an updated ED assessment according to the EFSA/ECHA ED GD. The applicants were therefore asked to provide one new combined ED assessment. In May 2020, two assessments of the endocrine disrupting potential of tebuconazole were submitted by the applicants (Bayer TF and EU Tebuconazole Task Force). The conclusions from these two reports were very similar and have been summarised below, followed by a comment from the DK-RMS.

Finally, the DK-RMS conducted the ED assessement in line with the EFSA/ECHA guidance for the identification of endocrine disruptors (2018). Please refer to annex II to the CLH report.

B.6.8.3.1.1. Additional studies related to ED adversity - not included in previous sections

Repeated dose (B.6.3.), chronic toxicity (B.6.5.), 2-generation reproductive toxicity (B.6.6.1.), developmental toxicity (B.6.6.2.), developmental neurotoxicity (B.6.6.2.1.2.) studies and relevant apical studies from the literature have been described in previous sections of this document and have not been re-presented here. Information on treatment-related adverse effects potentially related to ED from these studies (level 4 and 5 studies of the OECD Conceptual Framework (CF) on ED) are summarised in **Error! Reference source not found.**and discussed by UK-RMS immediately following the table. Additional apical studies informing on adversity and not evaluated in previous sections are presented below.

Male and female pubertal assay

Previous evaluation	None – submitted for the purpose of renewal (study owned by Bayer Task force)

Study ID	B.6.8.3.1.2/01
Report title	Assessment of pubertal development and thyroid function in juvenile/peripubertal male and female rats
Matrix ID	32
GLP	Yes
Guideline(s)	US-EPA OPPTS 890.1500, 890.1450 (2009)
Deviations	None.
Test material	Tebuconazole Batch N.o: K 689052 Purity: 97.5 %
Vehicle & controls	Aqueous solution of 0.5 % Methylcellulose 400 (Sigma Aldrich) and 0.4 % Tween 80 (Merck)
Species **Strain**	Rat Crl:CD (SD) Sprague Dawley
Administration	Oral gavage 0, 75 and 150 mg/kg bw/day 15/group Males: Application on 31 consecutive days (PND 23–53) Females: Application on 21 consecutive days (PND 22–42)
Acceptable	Acceptable as part of a weight-of-evidence approach
Result	Treatment-related adverse effects on endocrine organs (adrenals, pituitary and sexual organs) and hormones (testosterone) were seen from 75 mg/kg bw/d. There was also a delay in VO in females and PPS in males at the top dose.

Methods
Groups of 15 female rats, 22-days old, were exposed to tebuconazole by oral gavage for 21 days, from PND 22 to 42 Groups of 15 male rats, 23-days old, were exposed to tebuconazole by oral gavage for 31 days, from PND 23 to 53, the day of birth being PND 0. Doses given were 0, 75, and 150 mg/kg bw/day formulated in aqueous 0.5% methylcellulose 400 + tween 80. Clinical observations and body weight were recorded daily. Observations made included: Clinical signs, mortality, body weight and body weight change parameters, terminal body weight, vaginal opening (VO) and preputial separation (PPS), clinical chemistry and hormonal analysis, oestrous cyclicity parameters (mean age at first vaginal estrus, mean cycle length, cycling, regularly cycling), necropsy with organ weights (ovaries*, uterus*, testes*, epididymides*, seminal vesicles, ventral prostate gland, dorsolateral prostate, kidneys*, thyroid gland*, liver, adrenals, pituitary gland, levator ani plus bulbocavernosus muscle complex (LABC, significant macroscopic findings*) and histological examination (of organs marked with *)

Results
There was no mortality throughout the study. Treatment-related clinical signs of toxicity were confined to increased salivation in 9/15 males observed on several occasions throughout the study at 150 mg/kg bw/day and in 2/15 females observed on one or a few occasions throughout the study at both 75 and 150 mg/kg bw/day.

Table 6.8-6. Incidence of salivation

Sex	**Dose level (mg/kg bw/d)**					
	Vehicle Control (0)		**Low dose (75)**		**High dose (150)**	
	# observed	# examined	# observed	# examined	# observed	# examined
Male	0	15	0	15	9	15
Female	0	15	2	15	2	15

Males:
After an initial growth impairment, the growth of exposed animals was comparable to that of controls. Body weights at termination of the study were reduced with 8% by 150 mg/kg. There was a nominal (not statistically significant) 5% reduction in final body weight in the low dose group.

At 150 mg/kg bw/day, when compared to controls the mean body weight was statistically significantly reduced by

6 % to 10 % from Day 2 (PND 24) onwards. On Day 2, there was a statistically significant mean body weight loss of 1.5 g ($p \leq 0.01$) compared to a mean body weight gain of 4.9 g in controls. Evaluation of individual animal data reveal that on the first day of dosing 5 /15 males displayed weight loss (2 to 5 g equal to 5-10% of own bw), which was regained on the next day (up to 12 g bw increase equal to 20% of own bw). On Day 3 and 4 (PND 25 and 26, respectively) the mean cumulative body weight gain (including the initial weight drop) was statistically significantly reduced by 61 % ($p \leq 0.01$) and 33 % ($p \leq 0.01$), respectively, when compared to controls. On Day 8 and 10 (PND 30 and 32, respectively) the mean body weight gain per day was statistically significantly reduced by 30 % ($p \leq 0.01$) and 26 % ($p \leq 0.01$), respectively. Thereafter, the mean cumulative body weight gain remained statistically significantly reduced by 8 % to 28 % throughout the treatment, when compared to controls.

At 75 mg/kg bw/day, when compared to controls the mean body weight was statistically significantly reduced by 6 to 7 % on several occasions ($p \leq 0.05$). When compared to controls, the mean body weight gain per day was statistically significantly reduced by 53 % ($p \leq 0.05$) on Day 2 (PND 24). Thereafter, the mean body weight gain per day was comparable to controls except on Day 10 (PND 32, 18 % reduction, $p \leq 0.01$) and Day 27 (PND 49, 21 % reduction, $p \leq 0.01$) when compared to controls. The mean cumulative body weight gain was lower than in controls throughout the study, with a statistically significant reduction of between 7 % ($p \leq 0.05$) and 19 % ($p \leq 0.01$) from Day 3 to 25 (PND 25-47). Overall, there were effects on body weight and body weight gain in males from 75 mg/kg bw/day, becoming more severe at 150 mg/kg bw/day.

At 150 mg/kg bw/day, the age at PPS was statistically significantly increased to PND 42.60 ($p \leq 0.01$) when compared to PND 40.13 in controls. However, the mean body weight at PPS (PND 42) was not affected when compared to controls. At 75 mg/kg bw/day, no statistically significant effect on either PPS or complete PPS was observed when compared to controls. Overall, a delay in PPS was observed at the top dose.

Based on the marked body weight effect at 150 mg/kg bw/day at the start and throughout treatment, it is considered that the slight delay in PPS was the consequence of this effect. This is further supported by the absence of effects on the mean body weight at the time of PPS at both doses; in other words, the body-weight at the time of PPS was the same across groups, but, because of the offspring generalised toxicity, the time to reach this body-weight (and hence for PPS to occur) was delayed in the high-dose group.

Table 6.8-7 General growth and preputial separation

		Vehicle control		**Low dose [75 mg/kg bw/day]**		**High dose [150 mg/kg bw/day]**		**HCD** (mean and range)
		mean	SD/SE	mean	SD/SE	mean	SD/SE	
Body weight at weaning (PND 21;g)	U	58.1	5.0	57.2	5.3	57.7	5.3	59.6 (51.5 - 67.4)
Initial body weight (PND 23; g)	U	66.0	5.5	64.8	5.4	65.3	6.4	67.5 (57.2 - 75.4)
Final body weight (g)	U	311.4	18.7	295.7	19.9	**286.8***	29.1	316.8 (273.4 - 378.0)
Body weight gain (final – initial; g)	U	246.0	17.9	231.0	18.3	**226.0***	24.7	-
Age at PPS (PND)	U	40.13	1.8	41.33	1.7	**42.60****	1.8	41 (38 – 44)
	A	40.12	0.5	41.34	0.5	**42.60****	0.5	-
Age at complete PPS (PND)	U	48.00	4.3	49.93	3.8	50.33	4.0	45 (41 – 54)
	A	47.98	1.1	49.95	1.1	50.33	1.1	-
Body weight at PPS (g)	U	200.53	19.6	198.19	16.9	204.69	24.1	206.2 (179.4 - 249.9)
	A	199.77	4.9	198.95	4.9	204.68	4.9	-
Body weight at complete PPS (g)	U	267.68	40.5	266.31	28.7	262.09	38.7	249.6 (202.2 - 314.9)
	A	266.76	9.1	267.26	9.1	262.08	9.1	-

		Vehicle control		Low dose [75 mg/kg bw/day]		High dose [150 mg/kg bw/day]		HCD (mean and range)
		mean	SD/SE	mean	SD/SE	mean	SD/SE	

N = 15
HCD: Historical control data (2010 – 2011; 3 studies)
U: Unadjusted for body weight on PND 21
A: Adjusted for body weight on PND 21 (as recommended in OPPTS guideline)
SD: Standard Deviation / SE= standard error (for covariance analysis)
* Significantly different from controls at p 0.05
** Significantly different from controls at p 0.01

DK RMS has included the figure below in order to clarify the effect on male body weights. A slight drop in bw (<10%) is seen on the first day of dosing at the high dose, and subsequently the exposed animals follow growth curves parallel with the control group, but resulting in lower body weights

Table 6.8-8 Preputial separation

	Vehicle control	Low dose [75 mg/kg/day]	High dose [150 mg/kg/day]
Number of animals examined	15	15	15
Incidence (not separated)	0	0	0
Incidence (not completely separated)	3	4	7

Table 6.8-9 Organ weights in males at necropsy

		Vehicle control		Low dose [75 mg/kg/day]		High dose [150 mg/kg/day]	
		mean	SD/SE	mean	SD/SE	mean	SD/SE
Liver (g)	U	13.89	1.52	13.28	1.46	14.14	2.30
	A	13.85	0.456	13.33	0.456	14.14	0.455
	R	4.4540	0.3077	4.4873	0.3229	**4.9060****	0.3564
Kidneys (g)	U	2.39	0.15	2.22	0.14	2.17**	0.28
	A	2.39	0.047	2.23*	0.047	2.17**	0.047
	R	0.7693	0.0425	0.7533	0.0401	0.7560	0.0439
Pituitary (mg)	U	10.55	1.19	**9.65***	1.01	**9.27****	0.92
	A	10.55	0.274	**9.65***	0.274	**9.27****	0.274
	R	0.0034	0.0003	0.0033	0.0003	0.0033	0.0005
Adrenals (mg)	U	42.15	6.26	**34.41****	5.62	**36.57***	5.89
	A	41.96	1.451	**34.60****	1.451	**36.56***	1.449
	R	0.0136	0.0020	**0.0116****	0.0016	0.0128	0.0018
Seminal vesicle & coagulating gland, with fluid (mg)	U	594.1	161.8	468.9	174.2	398.7**	166.0
	A	593.6	43.86	469.3	45.33	**398.9****	47.00
Seminal vesicle & coagulating gland, without fluid (mg)	U	314.5	66.5	259.2	67.9	**242.2***	71.4
	A	313.8	17.85	259.9	17.86	**242.2***	17.83
Ventral prostate (mg)	U	249.0	52.1	230.4	48.1	225.1	57.8
	A	248.6	13.79	230.8	13.79	225.1	13.77
Dorsolateral	U	168.5	51.8	**132.8***	26.2	**133.4***	28.5

		Vehicle control		Low dose [75 mg/kg/day]		High dose [150 mg/kg/day]	
		mean	SD/SE	mean	SD/SE	mean	SD/SE
prostate (mg)	A	168.6	9.78	**132.7***	9.78	**133.4***	9.76
LABC (mg)	U	686.8	115.6	611.5	135.5	**522.2****	102.0
	A	687.1	31.00	611.3	31.00	**522.2****	30.96
Epididymis, left (mg)	U	228.4	20.9	223.3	19.8	**197.4****	27.7
	A	228.4	6.02	223.3	6.02	**197.4****	6.02
Epididymis, right (mg)	U	240.2	16.2	231.4	23.0	**209.7****	25.7
	A	240.3	5.75	231.3	5.75	**209.7****	5.74
Testis, left (mg)	U	1379.5	114.3	1363.0	130.5	1243.4	276.3
	A	1379.4	49.28	1363.2	49.28	1243.4	49.21
Testis, right (mg)	U	1401.8	97.9	1379.3	120.9	1245.8	288.8
	A	1399.9	49.24	1381.3	49.24	1245.8	49.17

N = 15
U: Unadjusted for body weight on PND 21
A: Adjusted for body weight on PND 21 (as recommended in OPPTS guideline)
R: Organ-to-body weight ratio (relative to body weight)
SD: Standard Deviation / SE= standard error (for covariance analysis)
LABC: levator-ani bulbocavernosus muscle
* Significantly different from controls at p 0.05
** Significantly different from controls at p 0.01

Females:
At 150 mg/kg bw/day, when compared to controls the mean body weight was statistically reduced by 7 % to 9 % from Day 2 to 11 (PND 23-32). On Day 2 (PND 23), there was a statistically significant body weight loss of 0.9 g ($p \leq 0.01$) compared to a body weight gain of 4.4 g in control animals. The mean body weight gain per day was statistically significantly reduced by 17 % ($p \leq 0.05$) to 28 % ($p \leq 0.01$) on Days 6 and 11 (PND 27 and 32, respectively) when compared to controls. The mean cumulative body weight gain was reduced by 71 % on Day 3 (PND 24, $p \leq 0.01$) and by 31 % on Day 4 (PND 25, $p \leq 0.01$) when compared to controls. From Day 5 to 15 (PND 26-36) the mean cumulative body weight gain was statistically significantly reduced by 11 % ($p \leq 0.01$) to 26 % ($p \leq 0.01$) when compared to controls. Thereafter it gradually became comparable to controls.

At 75 mg/kg bw/day, the mean body weight was comparable to control animals throughout the treatment period. The mean body weight gain per day was reduced by 73 % ($p \leq 0.01$) on Day 2 (PND 23) when compared to controls and from Day 3 (PND 24) onwards it was comparable to controls. The mean cumulative body weight gain was statistically significantly reduced by 11 % to 22 % ($p \leq 0.05$ or $p \leq 0.01$) from Day 3 to 7 (PND 24-28) and thereafter it gradually became comparable to controls. Overall there were effects on body weight gain in females from 75 mg/kg bw/day, becoming more severe at 150 mg/kg bw/day.

At 150 mg/kg bw/day, the age at VO and complete VO were statistically significantly delayed to PND 35.40 ($p \leq 0.05$) and PND 36.13 ($p \leq 0.01$), respectively, compared to PND 33.40 and PND 33.47 in controls. However, there was no effect on the body weight at VO (PND 35) or complete VO (PND 36) when compared to controls although a nominal increase could be observed. At 75 mg/kg bw/day, no effect on VO was observed when compared to controls but a nominal delay in VO was registered. Overall, a delay in VO was observed in females at the top dose of 150 mg/kg bw/day.

Based on the significant body weight effect at 150 mg/kg bw/day at the start and throughout treatment, it is considered that the slight delay in VO was the consequence of this effect. This is further supported by the absence of effects on the mean body weight at the time of VO at both doses; in other words, the body-weight at the time of VO was the same across groups, but, because of the offspring generalised toxicity, the time to reach this body-weight (and hence for VO to occur) was delayed in the high-dose group.

No treatment-related findings were observed in the thyroid gland of both males and females.

Table 6.8-10. General growth and vaginal opening

		Vehicle control		**Low dose [75 mg/kg bw/day]**		**High dose [150 mg/kg bw/day]**		**HCD** (mean and range)
		mean	SD/SE	mean	SD/SE	mean	SD/SE	
Body weight at weaning (PND 21; g)	U	56.4	4.0	56.6	4.0	56.7	4.4	56.6 (47.8 - 66.1)
Initial body weight (PND 22; g)	U	58.7	3.8	59.2	4.0	59.1	4.7	58.6 (50.3 - 67.5)
Final body weight (g)	U	168.3	12.3	168.9	12.5	164.0	14.3	166.7 (144.0 - 193.0)
Body weight gain (final – initial; g)	U	108.3	9.4	110.1	11.0	107.3	11.6	-
Age at vaginal opening (PND)	U	33.40	1.8	34.53	1.8	**35.40***	2.5	34 (30 - 40)
	A	33.39	0.5	34.54	0.5	**35.41***	0.5	-
Age at complete vaginal opening (PND)	U	33.47	1.8	34.67	1.8	**36.13****	3.2	34 (30 - 43)
	A	33.44	0.6	34.67	0.6	**36.15****	0.6	-
Proportion unopened (incidence)		0	NA	0	NA	1	NA	-
Body weight at vaginal opening (g)	U	122.34	12.4	127.14	14.4	128.32	20.4	121.2 (95.4 - 158.7)
	A	122.50	4.1	127.11	4.1	128.19	4.1	-
Body weight at complete vaginal opening (g)	U	122.90	13.2	128.23	15.0	132.33	20.7	122.3 (95.4 - 159.7)
	A	123.02	4.3	128.21	4.3	132.22	4.3	-

N = 15
HCD: Historical control data (2010 – 2011; 3 studies)
U: Unadjusted for body weight on PND 21
A: Adjusted for body weight on PND 21 (as recommended in OPPTS guideline)
SD: Standard Deviation / SE= standard error (for covariance analysis)
* Significantly different from controls at p 0.05
** Significantly different from controls at p 0.01
NA: Not applicable

At the dose of 150 mg/kg bw/day there was also increased liver weight, lower mean urea and total bilirubin in both sexes. In addition, in males, there were reduced adrenal and sexual organ weights (seminal vesicle, dorsolateral prostate, epididymis and LABC), reduced testosterone and macroscopic effects of sexual organs (atrophic/small prostate, seminal vesicle, LABC and testis) (Table 6.8-5a). The changes include prostate weight reduction of 21% at 75 mg/kg bw/d, a 33% reduction in seminal vesicle weight at 150 mg/kg bw/d and a 24% reduction in LABC weight at 150 mg/kg bw/d.

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Table 6.8-11. Macroscopic changes in males, scheduled sacrifice

INCIDENCE OF MACROSCOPIC CHANGES IN MALES, SCHEDULED SACRIFICE			
Dose level (mg/kg/day)	0	75	150
Atrophic/small prostate	1/15	2/15	5/15
Atrophic/small seminal vesicle	2/15	5/15	7/15
Atrophic/small LABC	0/15	1/15	3/15
Atrophic/small testis	1/15	2/15	3/15

Other changes were considered as incidental and not treatment-related.

The dose of 75 mg/kg bw/day caused a lower mean total bilirubin concentration in both sexes, a lower urea concentration in females and lower testosterone in males. At 150 mg/kg bw/d testosterone was reduced with 53%. Other effects at 75 mg/kg bw/day included an increased liver weight in females and decreased adrenal, pituitary gland, and dorsolateral prostate weights in males. Macroscopic findings at 75 mg/kg bw/day were confined to atrophic/small seminal vesicle.

Table 6.8-12. Selected hormone levels and clinical chemistry data

	Vehicle control		**Low dose 75 mg/kg bw/d**		**High dose 150 mg/kg bw/d**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Males						
Hormones						
Serum testosterone (pg/mL)	1769	1012	1464	785	**839***	817
Clinical chemistry						
Urea (mmol/mL)	5.14	0.42	4.87	0.64	4.61*	0.62
Total bilirubin (µmol/L)	0.4	0.2	**0.1****	0.1	**0.0****	0.1
Females						
Clinical chemistry						
Urea (mmol/mL)	4.58	0.67	3.89*	0.58	3.81**	0.73
Total bilirubin (µmol/L)	0.2	0.2	**0.0****	0.1	**0.0****	0.1

SD = standard deviation
* Significantly different from controls at $p \leq 0.05$
** Significantly different from controls at $p \leq 0.01$

Male pubertal-like study from the open literature

Previous evaluation	None – (open literature)

Study ID	B.6.8.3.1.2/02
Author(s)	Chen *et al.* (2019)

Study title and journal	Pubertal exposure to tebuconazole increases testosterone production via inhibiting testicular aromatase activity in rats. Chemosphere, 230, p 519-526
Matrix ID	56
Test substance	Tebuconazole
Purity (%) Batch no.	95%
GLP	No
Guideline	No
Deviation	n/a
Reliability	Reliable with restrictions: - Acceptable, well-documented study performed based on basic scientific principles - One shortcoming is that the the groupsize is n = 6
Relevance to hazard assessment	Relevant to mode of action analysis and endocrine activity

Methods
Male rats (n = 6) were exposed to tebuconazole for 21 days from PND 35-56 at doses of 0, 25, 50 and 100 mg/kg bw/day by oral gavage. Body weight, testis weight and epididymides were weighed and serum hormone levels of testosterone, progesterone, LH and FSH were measured. Leydig and sertoli cell numbers were evaluated in testis as was gene and protein expression in testis. CYP19A1 activity was evaluated in primary rat leydig cells.
Results and disussion
Tebuconazole exposure increased serum testosterone level but lowered estradiol level at a dose of 100mg/kg, without affecting serum luteinizing hormone and follicle-stimulating hormone concentrations. Tebuconazole up-regulated the expression of testicular Cyp11a1, Hsd11b1, and Fshr genes as well as their proteins at a dose of 100 mg/kg. However, tebuconazole did not stimulate the proliferation of Leydig cells. Tebuconazole in vitro inhibits aromatase activity in primary rat Leydig cells with IC50 value of 40 mmol/L. In conclusion, tebuconazole exposure stimulates pubertal Leydig cell differentiation via inhibiting aromatase activity.

No effect on gene expression of Lhcgr, Scarb1, Star, Hsd3b1, Cyp17a1, Hsd17b3, Dhh, Amh, Sox9

This study showed how aromatase inhibition resulted in effects on steroid hormone levels and changes in the developing testis of male rats exposed late pubertally to tebuconazole. This study found no effect on bw nor on epididymides and testis weight up to 100 mg/kg. This could be due to the rather late initiation of exposure (PND35)

Male 28-day like study from the open literature (1)

Previous evaluation	None – (open literature)

Study ID	B.6.8.3.1.2/03
Author(s)	Yang *et al.* (2018)
Study title and journal	Effects of tebuconazole on cytochrome P450 enzymes, oxidative stress, and endocrine disruption in male rats Environmental Toxicology, 33:899-907
Test substance	Tebuconazole (0, 10, 25, 50 mg/kg bw/d)
Purity (%) Batch no.	99.5%
GLP	No
Guideline	No
Deviation	n/a
Reliability	Reliable with restrictions: - Acceptable, well-documented study performed based on basic scientific principles
Relevance to	Relevant to mode of action analysis and endocrine activity

hazard assessment	

Methods
Male rats (n = 10) were orally exposed to tebuconazole for 28 days from 7 weeks (56 days) of age at doses of 0, 10, 25 and 50 mg/kg/bw/d.

Results and discussion
Serum testosterone levels were dose dependently decreased in 10, 25, and 50 mg/kg bw/d (29, 30 and 37%). No significant effect was seen on testicular testosterone levels, however, there was a trend towards decrease. Significant dose dependent decrease in cauda epididymal sperm count in all groups (11, 14 and 21 %). No effect on testicular spermatid count or weights of testis, epididymis, seminal vesicle, prostate, LABC, thyroid or adrenals.

Male 28-day like study from the open literature (2)

Previous evaluation	None – (open literature

Study ID	B.6.8.3.1.2/03
Author(s)	Schmidt *et al.* (2016)
Study title and journal	Effects of tebuconazole on cytochrome P450 enzymes, oxidative stress, and endocrine disruption in male rats Environmental Toxicology, 33:899-907
Test substance	Tebuconazole (1 ppm (0.06 mg/kg bw), 10 ppm (0.59±0.04 mg/kg bw), 100 ppm (6.61±0.83 mg/kg bw), 300 ppm (19.01±2.19 mg/kg bw), 1000 ppm (71.24±4.38 mg/kg bw).
Purity (%) Batch no.	96.2%
GLP	No
Guideline	No
Deviation	n/a
Reliability	Reliable with restrictions: - Acceptable, well-documented study performed based on basic scientific principles
Relevance to hazard assessment	Relevant to mode of action analysis and endocrine activity

Methods
Male rats (n = 5) were exposed via diet to tebuconazole for 28 days from 9 weeks of age at doses of (1 ppm (0.06 mg/kg bw), 10 ppm (0.59±0.04 mg/kg bw), 100 ppm (6.61±0.83 mg/kg bw), 300 ppm (19.01±2.19 mg/kg bw), 1000 ppm (71.24±4.38 mg/kg bw).

Results and discussion
There was no effect on body weight, adrenals weight, testis weight or prostate weight.

B.6.8.3.1.2. Endocrine activity

In this subsection, all the studies (regulatory and from the literature) which inform on endocrine activity (OECD ED CF level 2 and 3 studies) are considered.

***In vitro* assays**

ToxCast and Tox21 data

Previous evaluation	None – submitted for the purpose of renewal (study owned by Bayer Task force)

Study ID	B.6.8.3.1.4/01
Report title	Consideration of ToxCast and Tox21 Endocrine Activity Data for Tebuconazole
Matrix ID	61
Date	July 20, 2016
GLP	No
Guideline(s)	Not applicable
Deviations	Not applicable
Acceptable	Acceptable as part of a weight-of-evidence approach
Result	ToxCast/Tox21 data suggest that tebuconazole disrupts steroidogenesis *in vitro*. Negative for E, A and T activity.

Methods
ToxCast and Tox21 dose-response data were accessed from the MySQL database and downloaded as summary files (version invitrodb_v2, released October 2015). ER (oestrogen receptor) and AR (androgen receptor) AUC (area under curve) scores were accessed from the Endocrine Disruptor Screening Program for the 21st Century (EDSP21) Dashboard. The Interactive Chemical Safety for Sustainability Dashboard was also used to visualize the data.

Results and conclusion
Weak responses in two of 18 ER-associated assays and a negative result in the ER network model provide a strong mechanistic argument against a direct interaction of tebuconazole with estrogenic or anti-estrogenic pathways.

Table 6.8-13. Results of the ER AUC Model for tebuconazole

ER AUC Model Activity	Range of Positive Results	Tebuconazole Result	Conclusion
Agonist	0.1-1	0	Negative
Antagonist	0.1-1	0	Negative

ER – oestrogen receptor
AUC – area under curve

Although the AR antagonist score is positive, closer examination of the individual assay results for tebuconazole suggests that non-specific effects or cytotoxicity cannot be discounted.

Table 6.8-14. Results of the AR AUC Model for tebuconazole

AR AUC Model Activity	Range of Positive Results	Tebuconazole Result	Conclusion
Agonist	0.05-1	0.00194	Negative
Antagonist	0.05-1	0.122	Positive

AR – androgen receptor
AUC – area under curve

With regard to thyroid activity, tebuconazole was positive for one of four thyroid receptor-related assays. This positive result for TR antagonism is a loss of signal assay, and as the activity occurs in the cytotoxicity region, the signal loss could be attributed to an overall decrease in cell number rather than specific TR antagonism. The other assay for TR antagonism and both assays for TR agonism were negative. In addition, tebuconazole was negative in the one assay available to evaluate thyroperoxidase inhibition. Overall, given the inconsistency among assays and possible interference of cytotoxicity, it is unlikely that tebuconazole interacts directly with thyroid hormone receptors. However, these data indicate that increased hepatic catabolism could contribute to altered thyroid hormone homeostasis.

The ToxCast/Tox21 data suggest that tebuconazole disrupts steroidogenesis *in vitro*. ToxCast/Tox21 contains assays to measure 10 hormones and therefore monitor biosynthesis of both corticosteroids and sex steroid hormones. ToxCast/Tox21 also has two assays for direct measurement of aromatase inhibition. Tebuconazole was

positive in 8 out of 20 steroidogenesis assays, indicating decreased levels of 8 hormones (17β-estradiol, estrone, testosterone, 11-deoxycorticosterone, 17α-OH progesterone, androstenedione, cortisol, deoxycortisol). Tebuconazole was also positive for one of 2 assays for aromatase inhibition. The AC_{50} values for most of the steroidogenesis assays (all but estradiol and estrone) are below the lower bound of the cytotoxicity region.

Overall, the ToxCast/Tox21 data indicate that tebuconazole does not possess oestrogen (E) androgen (A) or thyroid (T) activity, but disrupts steroidogenesis *in vitro*.

B.6.8.3.1.4/01 Discussion and conclusion by DK-RMS:	Overall, the ToxCast data indicates that tebuconazole does not interact with ER or TR. DK-RMS finds that AR-antagonism cannot be excluded based on this set of data and notes that 8 studies in the open literature has found AR antagonism with IC50 in the range 1-30 µM (See Vol 1, section 2.10). The evidence showing that tebuconazole disrupts steroidogenesis is strong as is the evidence that it is an aromatase inhibitor.

ER transactivation activity

Previous evaluation	None – submitted for the purpose of renewal (study owned by Bayer Task force)

Study ID	B.6.8.3.1.4/02
Report title	Tebuconazole: evaluation in the *in vitro* (Hela-9903) oestrogen receptor transcriptional activation assay
Matrix ID	39
Date	01 December 2011
GLP	Yes
Guideline(s)	US-EPA, OPPTS Series 890, Test Guideline N°890.1300: Oestrogen Receptor Transcriptional Activation (Human Cell Line (HeLa-9903)), October 2009
Deviations	None
Test material	Tebuconazole Batch N.o: K 689052 Purity: 97.5 %
Vehicle & controls	Vehicle: 0.1 % dimethylsulfoxid (DMSO) Positive controls: 17ß-estradiol (E2) (Batch no.: 010M0142; 100 % purity), 17α-estradiol (Batch no.: 029K4124; 99.9 % purity), 17α-methyltestosterone (Batch no.: 039K0268; 100 % purity), Negative control: corticosterone (Batch no.: BCBB5955; ≥99.6 % purity)
Acceptable	Acceptable as part of a weight-of-evidence approach
Result	Negative for estrogenic activity

Methods
The objective of the study was to evaluate the ability of tebuconazole to function as an oestrogen receptor (ER) α ligand and activate an agonist response using the immortal human cell line, HeLa-9903. This cell line is stably transfected with the human ERα and a firefly luciferase gene which possesses an oestrogen-responsive element in its promoter sequence. Approximately 3h after seeding the cells into 96-well plates, the cells were exposed for 22 – 24 h to either the vehicle (DMSO), a reference substance (17β-estradiol, 17α-estradiol, 17α-methyltestosterone or corticosterone) or the test substance, tebuconazole. All chemicals were tested at seven concentrations in triplicate in two independent runs performed on different days. The appropriate concentration range of tebuconazole, 10^{-10} to 10^{-4} mol/l, was based on solubility in the vehicle and on cytotoxicity to the cells. At the end of the exposure period, the cells were lysed and the luminescence (directly proportional to the cytoplasmic luciferase concentration itself proportional to the quantity of the compound bound to the human ERα) was estimated using specific luminescence assays (luciferase based reporter gene assay).

Results and conclusions
Tebuconazole did not show estrogenic activity in this assay.

Table 6.8-15. Results for tebuconazole

Compound	RPCmax	PCmax	Class
First run			

Tebuconazole	- 0.2 %	10^{-10}M	Negative
Second run			
Tebuconazole	- 1.6 %	10^{-10}M	Negative

Table 6.8-16. Results for reference chemicals

Compound	logPC50	logPC10	logEC50	Hill slope
First run				
17β-estradiol	-10.5	-11.7	-10.5	0.8
17α-estradiol	-9.1	-11.9	-9.1	0.3
Corticosterone				
17α-methyltestosterone	20.7	-34.3		
Second run				
17β-estradiol	-10.7	-11.4	-10.7	1.2
17α-estradiol	-8.9	-10.1	-8.9	0.8
Corticosterone				
17α-methyltestosterone	-7.1	-9.4		

The two independent runs gave similar results and the reference compounds gave the expected responses (i.e. 17β-estradiol, 17α-estradiol, and 17α-methyltestosterone as positive and corticosterone as negative) (Tables 6.8-9 & 6.8-10). The maximum response for tebuconazole was less than 10 % of the positive control 17β-estradiol in both runs. Therefore, tebuconazole was negative in the human oestrogen receptor transcriptional activation assay.

AR binding assay

Previous evaluation	None – submitted for the purpose of renewal (study owned by Bayer Task force)

Study ID	B.6.8.3.1.4/03
Report title	Evaluation of tebuconazole in the androgen receptor binding assay
Matrix ID	38
Date	08 December 2011
GLP	Yes
Guideline(s)	US-EPA, OPPTS Series 890, Test Guideline N°890.1150: Androgen Receptor Binding (Rat Prostate Cytosol), October 2009
Deviations	None
Test material	Tebuconazole Batch No.: K689052 Purity: 97.5 %
Vehicle & controls	R1881 (purity: 98.83 %) – positive control Dexamethasone (purity: 98.9 %) – weak positive control Vehicle (for tebuconazole and positive control compounds): DMSO (<3.3 % of total assay volume).
Acceptable	Acceptable as part of a weight-of-evidence approach
Result	IC_{50} = 56 µM (17.2 mg/L) Tebuconazole was considered as a weak AR binder

Methods

The objective of the study was to evaluate the inhibition of androgen receptor (AR) binding of R1881 in rat ventral prostate cytosol by the test substance tebuconazole. This *in vitro* test method involved mixing cytosol, [^{3}H]-R1881 (radioligand), and test or control substances in a common reaction tube. The potential inhibitory effect of the test substance on AR binding of R1881 was evaluated by measuring the amount of [^{3}H]-R1881 (radioligand) bound to the cytosolic proteins. Tebuconazole, dissolved in DMSO, was tested at eight concentrations (10^{-10} to 10^{-3} M) in triplicate in three independent evaluations performed on different days. Positive controls, R1881 and dexamethasone (weak positive), were run concurrently in each evaluation; at concentrations of 10^{-11} to 10^{-7} M and 10^{-10} to 10^{-3} M respectively.

Results and conclusions
Three saturation binding experiments using the cytosol demonstrated that AR was present in reasonable concentrations and was functioning with appropriate affinity for the native ligand R1881.

The same cytosol (prostate cytosol from Sprague-Dawley rats) was used for the competitive binding experiments in the present study. A total of three competitive binding experiments were conducted and the amount of prostate cytosolic proteins used in each experiment was 0.48 mg/assay tube.

For the three evaluations, the IC_{50} for R1881 were determined to be 7.7×10^{-10}, 9.3×10^{-10} and 7.0×10^{-10} M. The IC_{50} values for dexamethasone for the three evaluations were determined to be 4.1×10^{-5}, 3.6×10^{-5} and 3.7×10^{-5}M. The RBA (relative binding affinity) values for dexamethasone in comparison to R1881 were 0.0019, 0.0026 and 0.0019 %.

Table 6.8-17. Competitive binding with the reference compounds

Run	R1881		Dexamethasone		
	IC_{50} (M)	**Log IC_{50}**	**IC_{50} (M)**	**Log IC_{50}**	**RBA (%)**
Competition #1	7.7×10^{-10}	-9.1	4.1×10^{-5}	-4.4	0.0019
Competition #2Bis*	9.3×10^{-10}	-9.0	3.6×10^{-5}	-4.4	0.0026
Competition #3	7.0×10^{-10}	-9.2	3.7×10^{-5}	-4.4	0.0019
All three competitions	**8.0×10^{-10}**	**-9.1**	**3.8×10^{-5}**	**-4.4**	**0.0021**

Note: Minor differences between the data presented in the above table and the corresponding figures are due to rounding-up of the data in the table.
*: A competitive binding experiment #2 was discarded, due to a technical problem during the assay, consequently a competitive binding experiment #2bis was performed.
RBA - relative binding affinity

The IC_{50} values for tebuconazole in the three evaluations were determined to be 5.4×10^{-5}, 5.9×10^{-5} and 5.5×10^{-5} M. The RBA values for tebuconazole in comparison to R1881 were 0.0014, 0.0016 and 0.0013 %.

Table 6.8-18. Competitive binding with tebuconazole

Run	Tebuconazole		
	IC_{50} (M)	**Log IC_{50}**	**RBA (%)**
Competition #1	5.4×10^{-5}	-4.3	0.0014
Competition #2Bis*	5.9×10^{-5}	-4.2	0.0016
Competition #3	5.5×10^{-5}	-4.3	0.0013
All three competitions	**5.6×10^{-5}**	**-4.3**	**0.0014**

Note: Minor differences between the data presented in the above table and the corresponding figures are due to rounding-up of the data in the table.
*: A competitive binding experiment #2 was discarded, due to a technical problem during the assay, consequently a competitive binding experiment #2bis was performed.
RBA - relative binding affinity

Based on the average results of the three competitive assays in which the data fit the non-linear regression model and on average the competitive curve crossed 50 % of the remaining R1881 bound at the two highest concentrations, tebuconazole was considered a weak AR binder.

Steroidogenesis assay (1)

Previous evaluation	None – submitted for the purpose of renewal (study owned by Bayer Task force)

Study ID	B.6.8.3.1.4/04
Report title	Evaluation of tebuconazole in the H295R steroidogenesis assay
Matrix ID	37

Date	10 November 2011
GLP	Yes
Guideline(s)	US-EPA, OPPTS Series 890, Test Guideline N°890.1550: Steroidogenesis (Human Cell Line –H295R) (October 2009)
Deviations	None
Test material	Tebuconazole Batch No.: AE F069623-01-18 (K689052) Purity: 97.5 %
Vehicle & controls	Vehicle: 0.1 % dimethylsulfoxid (DMSO) Positive controls: Forskolin (white powder, Batch no.: 109K5057V; 98 % purity) – for sex steroid hormone secretion stimulation Prochloraz (white powder, Batch no.: SZE6220X; 99.1 % purity) – for sex steroid hormone secretion inhibition
Acceptable	Acceptable as part of a weight-of-evidence approach
Result	Tebuconazole reduces the secretion of both testosterone and oestrogen in human cells *in vitro.* ↓ testosterone ≥1 µM (0.3 mg/L) ↓ estradiol ≥10 µM

Methods
The objective of the study was to evaluate the effect of tebuconazole on steroidogenesis using H295R cell cultures. Three independent evaluations of tebuconazole were conducted in which the cells were exposed to seven concentrations of the test substance (10^{-10} to 10^{-4} M) for 48h. The culture medium was then recovered and the concentrations of testosterone and estradiol were estimated using specific Enzyme ImmunoAssay kits (EIAs). An evaluation of the responsiveness of the H295R cells to two reference compounds known to interfere with steroidogenesis (forskolin and prochloraz) had previously been established. Furthermore, the continued responsiveness of the H295R cells to forskolin and prochloraz was confirmed in evaluations run concurrently with the present study. Proficiency determinations had previously been conducted by the technician conducting the present study, which indicated that the EC_{50} values for forskolin and prochloraz were within the recommended range given in the guideline.

Results and conclusions
Tebuconazole was not cytotoxic to the H295R cells nor did it interfere with the hormone EIA kits.

Tebuconazole had no effect on testosterone secretion when treatment was between 10^{-10} M and 10^{-7} M with the fold changes varying between 0.78 and 1.08 for the three evaluations. A significant concentration-related decrease was recorded between 10^{-6} M ($p \leq 0.05$) and 10^{-4} M ($p \leq 0.001$ for both 10^{-5} M and 10^{-4} M) with the overall fold-change being 0.68 at 10^{-6} M, 0.19 at 10^{-5} M and 0.02 at 10^{-4} M.

No effect on estradiol concentration was observed following treatment with tebuconazole between 10^{-10} M to 10^{-6} M whereas a complete inhibition was recorded at 10^{-5} M and 10^{-4} M in each of the three evaluations.

Table 6.8-19. Hormone concentrations (mean ± SD), % change and mean fold change relative to DMSO controls after 48h treatment with tebuconazole (overall data from three evaluations)

Tebuconazole concentration (M)	Testosterone Mean ± SD (pg/mL)	% change	Estradiol Mean ± SD (pg/mL)	% change
DMSO Control	9576.3 ± 1803.8	--	273.7 ± 69.3	--
10^{-10}	8537.4 ± 1470.3	-11	284.8 ± 79.5[B]	+4
10^{-9}	8679.0 ± 2084.2	-9	296.0 ± 55.7	+8
10^{-8}	8655.4 ± 1591.3	-10	245.3 ± 47.6	-10
10^{-7}	9137.3	-5	274.1	No change

	± 1238.5		± 47.4	
10^{-6}	6475.8 ± 1154.8*	-32	298.2 ± 71.1	+9
10^{-5}	777.4 ± 331.3***	-81	Complete inhibition[C]	Complete inhibition[C]
10^{-4}	231.8 ± 93.1[A]***	-98	Complete inhibition[C]	Complete inhibition[C]

Tebuconazole induced a statistically significant concentration-related reduction in testosterone secretion starting from 10^{-6} M, with the overall fold-changes being 0.68 ($p \leq 0.05$) at 10^{-6} M, 0.19 at 10^{-5} M ($p \leq 0.001$) and 0.02 at 10^{-4} M ($p \leq 0.001$). No effect on estradiol concentration was observed following treatment with tebuconazole between 10^{-10} M to 10^{-6} M whereas a complete inhibition was recorded at 10^{-5} M and 10^{-4} M in each of the three evaluations. These results show that tebuconazole disrupts steroidogenesis, reducing the secretion of both testosterone and oestrogen in human cells *in vitro*.

Steroidogenesis assay (2)

Previous evaluation	None – submitted for the purpose of renewal (study owned by Bayer Task force)

Study ID	B.6.8.3.1.4/05
Report title	Assessment of Tebuconazole and BCS-AG59816 (main mammalian metabolite of Tebuconazole) in the H295R steroidogenesis screen
Matrix ID	35
Date	08 February, 2017
GLP	Yes.
Guideline(s)	US-EPA OPPTS 890.1550
Deviations	None.
Test material	Tebuconazole and its main metabolite BCS-AG59816 Tebuconazole: K689052 BCS-AG59816: SES12252-4-2
Vehicle & controls	Vehicle: 0.1 % dimethylsulfoxid (DMSO) positive controls: Forskolin– for sex steroid hormone secretion stimulation Prochloraz– for sex steroid hormone secretion inhibition
Acceptable	Acceptable as part of a weight-of-evidence approach
Result	Both tebuconazole and its main metabolite, BCS-AG59816, markedly interfered with steroidogenesis: ↓ testosterone ≥1 µM (0.3 mg/L) ↓ estradiol ≥10 µM (3 mg/L) ↓ progesterone ≥30 µM ↓ cortisol ≥3 µM (0.9 mg/L)

Methods
Tebuconazole and its main mammalian metabolite (BCS-AG59816) were evaluated for effects on progesterone, testosterone, estradiol and cortisol secretions at identical concentrations (0.3, 1, 3, 10 and 30 µM). Evaluation of cytotoxicity and solubility in DMSO indicated that both test items did not induce cytotoxicity and were soluble up to 100 µM. Forskolin (1 µM) and prochloraz (0.1 µM) were included as reference controls. DMSO at 0.1 % was used as the vehicle control. Cells were exposed in triplicate for 48h. The concentrations of each hormone were determined using specific Enzyme ImmunoAssay kits.

Results and conclusions
The effects of tebuconazole and its main metabolite BCS-AG59816 on steroidogenesis in the H295R screen are given in Table 6.8-14 below.

Table 6.8-20. Effects of tebuconazole and its main metabolite BCS-AG59816 on steroidogenesis in the H295R

screen

Compound	Concentration (µM)	Mean pg/mL (% Control)			
		Progesterone	Testosterone	Estradiol	Cortisol
Tebuconazole	0	3246	5102	249	23036
	0.3	3064 (94)	4122 (81)	267 (107)	20570 (89)
	1	2611 (80)	3467 (68)	238 (95)	19089 (83)
	3	2890 (89)	2325 (46)	214 (86)	15186 (66)
	10	2851 (88)	879 (17)	<60 (0)	8240 (36)
	30	1529 (47)	181 (4)	<60 (0)	1362 (6)
BCS-AG59816	0	3595	5123	255	22932
	0.3	2924 (81)	3875 (76)	241 (95)	21509 (94)
	1	2582 (72)	3847 (75)	275 (108)	19649 (86)
	3	2402 (67)	2756 (54)	279 (109)	16909 (74)
	10	2247 (63)	1212 (24)	127 (50)	9358 (41)
	30	1682 (47)	388 (8)	<60 (0)	2499 (11)
DMSO	0	3595	5123	255	22932
Forskolin	1	5592 (156)	7544 (147)	4054 (1589)	77001 (336)
DMSO	0	3246	5102	249	23026
Prochloraz	0.1	5457 (168)	1287 (25)	186 (75)	18483 (80)

Overall, both tebuconazole and its main metabolite, BCS-AG59816, markedly interfered with steroidogenesis when tested in the H295R screen. The effects recorded for tebuconazole were initially observed at lower concentrations than those recorded for BCS-AG59816. A marked inhibition of all four hormones was induced by both test substances. For tebuconazole the effects on testosterone started at 1 µM whereas for the metabolite clear effects were not observed until 3 µM. A complete inhibition of estradiol secretion was observed at 10 & 30 µM tebuconazole and at 30 µM BCS-AG59816. Data recorded for tebuconazole in this study agree with the data from the previous study.

Steroidogenesis assay (3)

Study ID	B.6.8.3.1.4/06
Author(s)	Prutner *et al.,* 2013
Study title and journal	Effects of single pesticides and binary pesticide mixtures on estrone production in H295R cells. Archives of Toxicology, 87, 2201-2214.
Matrix ID	62
Test substance	Tebuconazole (and other pesticides)
Purity (%) **Batch no.**	97.5-99.5% Not specified
GLP	No
Guideline	No
Deviation	n/a
Reliability	Reliable with restrictions – non-standard test;
Relevance to hazard assessment	Relevant as mechanistic data in LoE evaluation
Result	Tebuconazole decreased estrone production indicating aromatase inhibition

Methods

H295R cells were cultured for 24 h in 0.01, 0.1, 0.3, 1, 3, 10, 30, 100 µM tebuconazole. Estrone levels were measured and cytotoxicity assessed.

Findings and conclusions

The estrone concentration was reduced in a dose-dependent manner from 3 µM and upwards indicating aromatase inhibition. No cytotoxicity was seen at any of the tested concentrations.

Steroidogenesis assay (4)

Study ID	B.6.8.3.1.4/07

Author(s)	Shen *et al.,* 2017
Study title and journal	Effects of fungicides on rat's neurosteroid synthetic enzymes. BioMed Research International, Volume 2017, Article ID 5829756, 8 pages.
Matrix ID	63
Test substance	Tebuconazole (and other fungicides)
Purity (%) **Batch no.**	Not specified Not specified
GLP	No
Guideline	No
Deviation	n/a
Reliability	Reliable with restrictions – non-standard test; no purity reported
Relevance to hazard assessment	Relevant as mechanistic data in LoE evaluation
Result	Tebuconazole inhibits the activity of acitivity of 5a-reductase 1, 3 a- HSD, and retinol dehydrogenase 2.

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Methods

COS-1 cells were transfected with Akr1c14, Srd5a1 and RDh2 genes and 24 h after transfection proteins (5α-Red1, 3α-HSD, RDH2) were isolated for enzyme activity measurements. Proteins were incubated with tebuconazole (100 µM) for 60 minutes.

Findings and conclusions

The resuls showed inhibition of the enzyme 5α-Red1, which converts testosterone to DHT (IC50 = 8.670 µM). 3α-HSD, which converts DHT to DIOL was also inhibited (approximately 50% of control) and the enzyme retinol dehydrogenase (RDH2,) which converts DIOL to DHT, was also inhibited (approximately 50% of control).

Aromatase inhibition, 3βHSD inhibition, steroidogenesis

Study ID	B.6.8.3.1.4/08
Author(s)	Cao *et al.,* 2017
Study title and journal	The effects of fungicides on human 3β-hydroxysteroid dehydrogenase 1 and aromatase in human placental cell line JEG-3. Pharmacology, 100, 139-147.
Matrix ID	64
Test substance	Tebuconazole (and other fungicides)
Purity (%) **Batch no.**	Not specified Not specified
GLP	No
Guideline	No
Deviation	n/a
Reliability	Reliable with restrictions – non-standard test; no information on purity, no positive control included.
Relevance to hazard assessment	Relevant as mechanistic data in LoE evaluation
Result	Tebuconazole inhibited CYP19A1 activity, decreased progesterone production, decreased estradiol synthesis. No effect was seen on HSD3B1 activity.

Methods

The human placental cell line JEG-3 was used for investigations. A microsomal preparation was used for aromatase inhibition assay, a cell homogenate for 3β-HSD inhibition assay and intact cells for evaluation measurement of progesterone and estradiol concentrations. A test concentration of 100 µmol/l tebuconazole was used.

Findings and conclusions

CYP19A1 activity was inhibited with more than 50% (non-competitive inhibition), but no effect as seen HSD3B1 activity. Exposure also decreased progesterone production with more than 50% and estradiol synthesis (from DHEA) was reduced by more than 50 %. No cytotox was seen.

Aromatase assay (1)

Previous evaluation	None – submitted for the purpose of renewal (study owned by Bayer Task force)

Study ID	B.6.8.3.1.4/09
Report title	Evaluation of tebuconazole in the aromatase assay.
Matrix ID	36
Date	09 December 2011.
GLP	Yes.
Guideline(s)	US-EPA, OPPTS 890.1200 82009.
Deviations	None.
Test material	Tebuconazole Batch No.: AE F069623-01-18 (K689052) Purity: 97.5 %
Vehicle & controls	vehicle control: DMSO positive control: Formestane (Sigma, Batch no. 081K2133, purity: 99.6 %)
Acceptable	Acceptable as part of a weight-of-evidence approach.
Result	Tebuconazole inhibited the aromatase enzyme. IC_{50} = 1.95 µM (~0.6 m/L)

Methods
The objective of the study was to determine if tebuconazole could affect steroidogenesis by inhibiting the catalytic activity of aromatase (the enzyme responsible for the conversion of androgen to oestrogen). Enzyme activity was quantified by measuring the tritiated water (^3H_2O) by-product released during the aromatase reaction when incubating the enzyme source with radio-labelled androstenedione. The assay was performed using recombinant microsomes containing human aromatase and cytochrome P450 reductase. Competitive inhibition of aromatase by tebuconazole was determined by evaluating increasing serial concentrations of the test substance. Concurrent positive control evaluations were conducted to confirm the responsiveness of aromatase to formestane (4-hydroxyandrostenedione, a specific aromatase inhibitor).

Results and conclusions
Data for the background activity controls were within the guideline recommendations. No assay drift was recorded in the first and second run; however a marginal assay drift was recorded for the third run as the % changes for the full activity controls were between 110.6% and 89.4% instead of between 110% and 90%. The average aromatase activity in the full activity controls was 0.3058 ± 0.0346 nmol/mg protein/min.

Table 6.8-21. Effect of formestane on aromatase activity (% control)

Effect of formestane on aromatase activity (% control) from independent runs					
Concentration (Log M)	**no. of runs**	**Overall mean**	**Overall SD**	**Overall SEM**	**Overall % CV**
-5	3	0.9	0.25	0.14	28 %
-6	3	7.8	1.10	0.63	14 %
-6.5	3	18.6	2.01	1.16	11 %
-7	3	40.3	4.15	2.40	10 %
-7.5	3	65.7	3.20	1.85	5 %
-8	3	79.1	5.59	3.23	7 %
-9	3	96.8	2.92	1.69	3 %
-10	3	95.9	3.95	2.28	4 %

Note: Data have been rounded-up.
SD: standard deviation
SEM: standard error (SD/ sq root of n, where n = number of runs conducted)

Effect of formestane on aromatase activity (% control) from independent runs					
Concentration (Log M)	**no. of runs**	**Overall mean**	**Overall SD**	**Overall SEM**	**Overall % CV**

CV: coefficient of variation ((SD/mean)*100)

Table 6.8-22. IC_{50} of formestane

Formestane	log IC_{50}	IC_{50}	Hill Slope
1st Run	-7.3	5.06 x 10^{-8} M	-0.85
2nd Run	-7.1	7.32 x 10^{-8} M	-0.85
3rd Run	-7.2	6.49 x 10^{-8} M	-0.93
Overall	**-7.2**	**6.25 x 10^{-8} M**	**-0.88**

Note: Minor differences between the data presented in the above table and the corresponding graphs are due to rounding-up of the data in the table.

Formestane: The data generated confirmed the responsiveness of the aromatase enzyme to a reference inhibitor and indicated that all guideline criteria had been met for the positive control. Aromatase activity averaged 0.2925 ± 0.0210 nmol/mg protein/min at 10^{-10}M and 0.0028 ± 0.0005 nmol/mg protein/min at 10^{-5}M. The average slope of the concentration response curve was -0.88. Overall, the log IC_{50} was -7.2, which equated to an IC_{50} of 6.25 x 10^{-8}M.

Table 6.8-23. Effect of tebuconazole on aromatase activity (% control)

Effect of tebuconazole on aromatase activity (% control) from independent runs					
Concentration (Log M)	**no. of runs**	**Overall mean**	**Overall SD**	**Overall SEM**	**Overall % CV**
-3	3	0.1	0.32	0.19	320 %
-4	3	2.3	0.31	0.18	14 %
-5	3	19.3	1.63	0.94	8 %
-6	3	61.9	4.94	2.9	8 %
-7	3	89.9	3.74	2.2	4 %
-8	3	91.3	6.58	3.7	7 %
-9	3	86.0	4.65	2.7	5 %
-10	3	89.2	9.90	5.7	11 %

Note: Data have been rounded-up.
SD: standard deviation
SEM: standard error (SD/ sq root of n, where n = number of runs conducted)
CV: coefficient of variation ((SD/mean)*100)

Table 6.8-24. IC_{50} of tebuconazole

Tebuconazole	log IC_{50}	IC_{50}	Hill Slope
1st Run	-5.8	1.58 x 10^{-6} M	-1.02
2nd Run	-5.7	2.08 x 10^{-6} M	-0.93
3rd Run	-5.7	2.22 x 10^{-6} M	-1.01
Overall	**-5.7**	**1.95 x 10^{-6} M**	**-0.98**

Tebuconazole: The data generated indicated that this test substance inhibited the aromatase enzyme. Aromatase activity for tebuconazole averaged 0.2706 ± 0.0068 nmol/mg protein/min at 10^{-10}M and 0.0004 ± 0.0009 nmol/mg protein/min at 10^{-3}M. The overall log IC_{50} was -5.7 which equated to an overall IC_{50} of 1.95 x 10^{-6}M. The average slope of the concentration response curve was -0.98.

Aromatase assay (2)

Study ID	B.6.8.3.1.4/10
Study title and journal	Induction and inhibition of aromatase (CYP19) activity by various classes of pesticides in H295R human adrenocortical carcinoma cells. Toxicology and Applied Pharmacology, 182, 44-54.
Matrix ID	65

Test substance	Tebuconazole (and other azoles and pesticides)
Purity (%) **Batch no.**	Not specified Not specified
GLP	No
Guideline	No
Deviation	n/a
Reliability	Reliable with restrictions – non-standard test; purity not reported, cytotoxicity close to effective dose
Relevance to hazard assessment	Relevant as mechanistic data in LoE evaluation
Result	Tebuconazole inhibited aromatse activity at concentrations where initial cytotoxicity was seen.

Methods
H295R cells were used to investigate aromatase activity. A positive control for induction (8Br-cAMP) and a positive control for inhibtion (4- hydroxyandrostenedione) were included. Tested concentrations not reported, but estimated from graph to be 0.01-100 µM.

Findings and conclusions
CYP19 (aromatase) activity was inhibited with and IC50 of 50 µM. However, initiating cytotoxicity was seen at this concentration, and the effects seen can therefore be due to cytotoxicity rather than aromatase inhibition.

ER Binding

Previous evaluation	None – publication submitted for the purposes of renewal

Study ID	B.6.8.3.1.4/11
Author(s)	Laws *et al*., 2006
Study title and journal	Nature of the binding interaction for 50 structurally diverse chemicals with rat oestrogen receptors. Toxicological Sciences, 94(1), 46-56.
Matrix ID	66
Test substance	Tebuconazole
Purity (%) **Batch no.**	Not specified Not specified
GLP	No
Guideline	No
Deviation	n/a
Reliability	Unreliable – no primary information has been provided by the applicants
Relevance to hazard assessment	Relevance cannot be assessed as the study is unreliable
Result	Tebuconazole does not bind to the oestrogen receptor

Tebuconazole has been tested in the DSSTox (KIERBL) EPA Oestrogen Receptor Ki Binding assay (no further details provided by the applicants). It is concluded that tebuconazole does not bind to the oestrogen receptor.

Progesterone secretion in vitro (1)

Previous evaluation	None – publication submitted for the purposes of renewal

Study ID	B.6.8.3.1.4/12
Author(s)	Rieke *et al.,* 2014
Study title and	Combination effects of (tri)azole fungicides on hormone production and xenobiotic

journal	metabolism in a human placental cell line. International Journal of Env Research and Public Health, 11(9), 9660-9679.
Matrix ID	67
Test substance	Tebuconazole (and other azole fungicides)
Purity (%) **Batch no.**	Not specified – analytical grade SZBB055XV
GLP	No
Guideline	No
Deviation	n/a
Reliability	Reliable with restrictions – non-standard test; no information on purity; shortcoming in reporting; unclear statistical analysis
Relevance to hazard assessment	Relevant as mechanistic data in a WoE approach
Result	Tebuconazole decreased progesterone production

Methods
The human placental cell line Jeg-3 was incubated with tebuconazole (in DMSO) at concentrations ranging from 0.01 to 40 µM for 48 hours. Following treatment, cell viability, synthesis of steroid hormone production (progesterone and estradiol) and gene expression of steroidogenic and non-steroidogenic cytochrome-P-450 (CYP) enzymes were investigated. In addition, in order to evaluate whether the induction of CYP1A1 was AhR dependent, the ability of tebuconazole to activate the AhR was investigated using a reporter gene assay.

Findings and conclusions
A significant decrease in progesterone secretion was observed from 15 µM. No decreased cell viability was observed in the tested concentration range, indicating specificity of the observed effects. There were no effects on estradiol production and no effects on steroidogenic-dependent CYP19 mRNA levels. However, a clear dose response effect on the induction of CYP1A1, a CYP-enzyme important for xenobiotic metabolism was evident from 3 µM. However, if the cells were pre-incubated with a specific AhR inhibitor, CYP1A1 induction was suppressed. Tebuconazole was not able to activate the AhR.

Overall, in this non-standard *in vitro* test in human placental cells, tebuconazole decreased progesterone production.

Progesterone secretion in vitro (2)

Study ID	B.6.8.3.1.4/13
Author(s)	Atmaca *et al.,* 2018
Study title and journal	Effects of mancozeb, metalaxyl and tebuconazole on steroid production by bovine luteal cells in vitro. Envrionmetnal Toxicology and Pharmacology, 59, 114-118.
Matrix ID	68
Test substance	Tebuconazole (and macozeb and metalaxyl)
Purity (%) **Batch no.**	Not specified Not specified
GLP	No
Guideline	No
Deviation	n/a
Reliability	Reliable with restrictions – non-standard test; no information on purity
Relevance to hazard assessment	Relevant as mechanistic data in LoE evaluation
Result	Tebuconazole decreased progesterone production

Methods
Bovine midcycle corpus luteum were collected immediately after slaughter, dissociated into single cell supension and incubated with tebuconazole at concentrations 1, 10, 100 µM for 96h. Cell attachment, cell growth, cell-to-

cell contact and protesterone concentrations were investigated.

Findings and conclusions
A dose-dependent effect was seen with reductions of 15, 36 and 65% in progesterone synthesis on day 3 and 5 of incubation. The ffects were significant at 10 and 100 µM.

AR activity (1)

Study ID	B.6.8.3.1.4/14
Author(s)	Vinggaard *et al.,* 2008
Study title and journal	Screening of 397 chemicals and development of a quantitative structure-activity relationship model for androgen receptor antagonism. Chem. Res. Toxicol, 21, 813-823.
Matrix ID	69
Test substance	Tebuconazole (and other types of chemicals)
Purity (%) **Batch no.**	Not specified Not specified
GLP	No
Guideline	No
Deviation	n/a
Reliability	Reliable with restrictions – non-standard test; no purity reported
Relevance to hazard assessment	Relevant as mechanistic data in LoE evaluation
Result	Tebuconazol

Methods
AR transactivation was tested in a luciferase reporter assay (transfect CHO K1 cells). The cells were incubated with 1, 3, 10 or 30 µM for 20 h together with R1881 (0.1 nM). The response of R1881 (0.1 nM) was considered a 100 % response, and AR antagonism was measured as the reduction in this response. Cytotoxicity was evaluated.

Findings and conclusions
Tebuconazole showed AR-antagonistic response with an IC25 in the range 1-3 µM. No cytotoxicity was seen in this range. The data on tebuconazole was part af a large screening study for building a QSAR model and details on the results are therefore few.

AR activity (2)

Study ID	B.6.8.3.1.4/15
Author(s)	Orton *et al.,* 2011
Study title and journal	Widely used pesticides with previously unknown endocrine activity revealed as in vitro antiandrogens. Environmental Health Perspectives, 119, 794-800.
Matrix ID	70
Test substance	Tebuconazole (and other pesticides)
Purity (%) **Batch no.**	>97% Not specified
GLP	No
Guideline	No
Deviation	n/a
Reliability	Reliable with restrictions – non-standard test;
Relevance to hazard assessment	Relevant as mechanistic data in LoE evaluation
Result	Tebuconazol show anti-androgenic properties

Methods
Androgenic and anti-androgenic properties of tebuconazole was investigated a reporter gene assay (MDA-kb2 cells). For test of androgenicity the cells were incubated with tebuconazole only and for test of anti-androgenicity

the cells were incubated with tebuconazole and DHT for 24 h. The activity of the tebuconazole + DHT treated cells was compared to DHT alone (DHT considered 100% response). The tested reange was evaluated from the graph to be 0.1-40 µM. Cytotoxicity was evaluated.

Findings and conclusions
Tebuconazole did not show any androgenic properties, but showed strong AR antagonistic properties with IC20 = 2.89 µM. Cytotoxicity was seen at IC20 = 38.9 µM, meaning that the anti-androgenic response was not due to cytotoxicity. The positive control was procymidone with IC50 = 0.53 µM. An estimation of IC50 fro tebuconazole from the graph gives a value of approximately 8-10 µM.

AR activity (3)

Study ID	B.6.8.3.1.4/16
Author(s)	Christen *et al.,* 2014
Study title and journal	Additive and synergistic antiandrogenic activities of mixtures of azol fungicides and vinclozolin. Toxicology and Applied Pharmacology, 279, 455-466.
Matrix ID	71
Test substance	Tebuconazole (and other azole fungicides and vinclozolin)
Purity (%) **Batch no.**	Not specified Not specified
GLP	No
Guideline	No
Deviation	n/a
Reliability	Reliable with restrictions – non-standard test; no information on purity
Relevance to hazard assessment	Relevant as mechanistic data in LoE evaluation
Result	Tebuconazol showed anti-androgenic properties

Methods
Anti-androgenic properties of tebuconazole was investigated a reporter gene assay (MDA-kb2 cells). The cells were incubated with tebuconazole (co-incubation with DHT) for 24 h. The activity of the tebuconazole + DHT treated cells was compared to DHT alone (DHT considered 100% response).

Findings and conclusions
Tebuconazole showed strong AR-antagonistic response with maximal inhibition of 90% compared to the DHT response and an EC50 =6.86 µM. No cytotoxicity was seen in the concentration range tested.

AR activity (4)

Study ID	B.6.8.3.1.4/17
Author(s)	Lv *et al.,* 2017
Study title and journal	Effects of triazole fungicides on androgenic disruption and CYP3A4 enzye activity. Envrionmetnal Pollution, 222, 504-512.
Matrix ID	72
Test substance	Tebuconazole (and other triazole fungicides)
Purity (%) **Batch no.**	Not specified Not specified
GLP	No
Guideline	No
Deviation	n/a
Reliability	Reliable with restrictions – non-standard test; no information on purity
Relevance to hazard assessment	Relevant as mechanistic data in LoE evaluation
Result	Tebuconazol showed anti-androgenic properties as well as inhibition of CYP3A4

Methods
Androgenic and anti-androgenic properties of tebuconazole was investigated in a two-hybrid recombinant human androgen receptor (AR) yeast bioassay as well as inhibition of enzymatic activity of CYP3A4 by P450-Glo™ CYP3A4 bioassay. For the androgenic assay DHT was used as positive control, and the anti-androgenic assay was performed with co-incubation with DHT. For the CYP3A4 assay ketoconazole was used as positive control.

Findings and conclusions
Tebuconazole did not show any androgenic properites, however, anti-androgenic properties were registered with and IC50 = 9.34 µM (no positive control included here). For CYP3A4 inhibition the IC50 = 0.81 µM, the positive control ketoconazole had an IC50 = 0.2 µM. A correlation between anti-androgenic and CYP3A4 inhibition was also seen ($R^2 = 0.83$), which is of interest as interference with CYP3A4 may affects is metabolism of testosterone. No cytotoxic effects were registered.

AR activity and steroidogenesis (1)

Previous evaluation	None – publication submitted for the purposes of renewal

Study ID	B.6.8.3.1.4/18
Author(s)	Kjaerstad *et al.*, 2010a
Study title and journal	Mixture effects of endocrine disrupting compounds *in vitro*. International Journal of Andrology, 33(2), 425-433.
Matrix ID	73
Test substance	Tebuconazole (and other azole fungicides)
Purity (%) **Batch no.**	98% SZBB055XV
GLP	No
Guideline	No
Deviation	n/a
Reliability	Reliable with restrictions – non-guideline; shortcoming in reporting; stability not assessed. Inconsistency in effect concentration levels between this study and subsequent study by the same authors for the AR transactivation assay.
Relevance to hazard assessment	Relevant as mechanistic data in a WoE approach
UK-RMS Result	Antagonistic activity on AR – inconsistent with findings from other studies; Inhibition of testosterone production *in vitro*
DK-RMS result	Antagonistic activity on AR – in line with other studies such as Rouquie 2011 and Kjaerstad et al., 2010b

Methods
Tebuconazole (in DMSO) was tested in an AR transactivation assay in CHO cells at concentrations ranging from 0.025 to 50 µM and in a steroidogenesis assay in the H295R cell line at concentrations ranging from 0.5 to 30 µM for 48 hours.

Findings and conclusions
Tebuconazole had antagonistic activity on the AR from 0.5 µM. In addition, tebuconazole inhibited testosterone production in the H295R cell line from 0.1 µM and oestradiol production from 1 µM. The effects on testosterone production are consistent with the findings of other steroidogenesis assays, but the anti-androgenic activity is at odds with the results of other similar studies.

B.6.8.3.1.4/02 Discussion and conclusion by DK-RMS:	This study finds AR antagonistic properties of tebuconazole and that tebuconazole disrupts steroidogenesis. The AR-activity is in line with the results of other studies as Rouquie 2011 and 2 ToxCast assays but not all assays in ToxCast.

AR activity and steroidogenesis (2)

Study ID	B.6.8.3.1.4/19
Author(s)	Roelofs *et al.,* 2014
Study title and journal	Conazole fungicides inhibit Leydig cell testosterone secretion and androgen receptor activation in vitro. Toxicology Reports, 1, 271-283.
Matrix ID	74
Test substance	Tebuconazole (and other azole fungicides)
Purity (%) **Batch no.**	99.6% Not specified
GLP	No
Guideline	No
Deviation	n/a
Reliability	Reliable with restrictions – non-standard test
Relevance to hazard assessment	Relevant as mechanistic data in LoE evaluation
Result	Tebuconazole decreased testosterone production and showed AR antagonism.

Methods
Murine Leydig cells (MA-10 cells) were exposed to 0.3-10 µM of tebuconazole in combination with luteinzing hormone (10 ng/ml = 8.5 IU/ml, induces testosterone secretion) for 48 h. Testosterone levels were measured. Cytotoxicity was investigated at 10 uM.
Human T47D-ARE cells (AR reporter gene assay) were exposed to 10 pM-100 µM of tebuconazole to investigate androgen receptor agonism and antagonism. Flutamide was used as a positive control.

Findings and conclusions
Tebuconazole significantly reduced LH induced testosterone secretion with an IC50 of 9.34 µM. In the human T47D-ARE cells no AR agonism was seen, however, AR antagonism was seen with IC50 = 25.5 µM. The positive control (flutamide) had IC50 = 7.0 µM. An anti-androgenic effect of tebuconazole exposure was seen, both affecting testosterone concentration and showing AR antagonism.

AR activity, ER activity and steroidogenesis assay

Previous evaluation	None – publication submitted for the purposes of renewal

Study ID	B.6.8.3.1.4/20
Author(s)	Kjaerstad *et al.,* 2010b
Study title and journal	Endocrine disrupting effects *in vitro* of conazole antifungals used as pesticides and pharmaceuticals. Reproductive Toxicology, 30(4), 573-582.
Matrix ID	75
Test substance	Tebuconazole (and other azole fungicides)
Purity (%) **Batch no.**	98% C1717800
GLP	No
Guideline	No
Deviation	n/a
Reliability	Reliable with restriction – non-GLP or guideline; shortcoming in reporting; stability not assessed; uncertainty about statistical analysis.
Relevance to hazard assessment	Relevant as mechanistic data in a WoE approach
Result	Anti-estrogenic;

	Anti-androgenic; Disrupts steroidogenesis

Methods
Tebuconazole (in DMSO) was tested in an AR transactivation assay in CHO cells at concentrations ranging from 0.025 to 50 µM; in a steroidogenesis assay in the H295R cell line at concentrations ranging from 0.1 to 30 µM for 48 hours; and in a cell proliferation assay in MCF-7 at concentrations ranging from 0.001 to 150 µM .

Findings and conclusions
In the MCF-7 cell proliferation assay conducted in the presence of estradiol, tebuconazole had an anti-estrogenic effect from 1.6 µM. As cytotoxicity was detected at much higher concentrations, the decreased cell proliferation observed was considered a specific response. In the same assay conducted in the presence of testosterone, tebuconazole inhibited testosterone-induced proliferation ~~(anti-androgenic)~~ from 10 µM indicating CYP19 inhibition and/or other anti-estrogenic mechanism.

In the AR transactivation assay, tebuconazole had antagonistic activity from 3.1 µM. Cytotoxicity was observed only at the top concentration of 50 µM.

In addition, tebuconazole reduced testosterone (≥ 0.1 µM) and estradiol (≥ 3 µM) production and increased progesterone concentration (at 10 µM) in the H295R cell line.

The authors suggest that the critical endocrine mechanism for tebuconazole seems to be inhibition of androgen biosynthesis since this effect occurs at lower concentrations than the anti-estrogenic effects in the MCF-7 cell assay as well as the antagonizing effects on the AR. The effects on testosterone biosynthesis indicate an inhibition of enzymes involved in the conversion of progesterone to testosterone and might, at least partly, be due to inhibition of 17alpha-hydroxylase/17,20-lyase (CYP17).

***In vivo* assays**

Uterotrophic assay

Previous evaluation	None – submitted for the purpose of renewal (study owned by Bayer Task force)

Study ID	B.6.8.3.1.4/21
Study title	Tebuconazole - Evaluation in the immature rat - Uterotrophic assay
Matrix ID	33
Test substance	Tebuconazole
Purity (%) **Batch no.**	97.5 K689052
Vehicle	Aqueous solution of 0.5 % methylcellulose 400 + 0.5 % Tween 80
Positive control	Ethynyl estradiol at 0.001 mg/kg bw/day
GLP	Yes
Guideline	OECD Test Guideline No. 440 (2007)
Deviation	None.
Species/ **Strain/** **Sex/** **Group**	Rat Crl:CD (SD) Sprague Dawley Female, immature 6/group
Administration	Oral, gavage 3 consecutive days
Dose (mg/kg bw/day	0, 35, 75, 150
Acceptable	Acceptable as part of a weight-of-evidence approach.
Result	No evidence of an estrogenic potential of tebuconazole.

Methods
This study was conducted according to the OECD guideline 440 (2007) and US-EPA OPPTS Series 890, Test Guideline N° 890.1600 (2009). The objective of this study was to evaluate in a short term screening test (the

Immature Uterotrophic Bioassay) the estrogenic potential of tebuconazole. This was done by evaluating uterine weights in immature Sprague-Dawley female rats following exposure to tebuconazole using the oral route of administration (gavage) in an aqueous vehicle. Groups of six 19-day old Sprague-Dawley female rats were administered tebuconazole in aqueous formulation of 0.5% methylcellulose 400 + 0.4% Tween 80 by gavage for 3 days at dose levels of 0, 35, 75 and 150 mg/kg bw/day. Another group received ethynyl estradiol, a well-known potent oestrogen, at 1 µg/kg/day (positive control). The volume of administration was 5 mL/kg based on the concurrent daily measures of individual body weight. All animals were observed for mortality and clinical signs daily and body weights were recorded daily. At scheduled sacrifice, approximately 24 hr after administration of the last dose, uterine weights (wet and blotted) were recorded.

Results

There was no mortality observed during the course of the study. At 150 mg/kg bw/day, treatment related clinical signs consisted of reduced motor activity observed on Study day 3 in 5/6 animals. No treatment-related clinical signs were observed with ethynyl estradiol or tebuconazole up to the dose of 75 mg/kg bw/day throughout the course of the study.

At 150 mg/kg bw/day, mean body weight was reduced by 24 % on Day 3 ($p \leq 0.01$) compared to the controls. Overall, there was a mean body weight loss of 3 g during the treatment period, compared to a body weight gain of 8.2 g in the control group ($p \leq 0.01$). At 75 mg/kg bw/day, mean body weight was reduced by 8 % on Day 3 (not statistically significant) compared to the controls. Overall, the mean cumulative body weight gain was reduced by 38 % during the treatment period, compared to the controls ($p \leq 0.05$). At 35 mg/kg/day mean body weight and body weight changes were unaffected by treatment. The body weight of animals treated with Ethynyl estradiol (EE) was slightly reduced by 5 % on Day 3 (not statistically significant) compared to the controls, corresponding to a reduced body weight gain of 7 % during the treatment period.

Table 6.8-25. Group body weights (g) and cumulative body weight gains (g)

	Vehicle		**Tebuconazole**						**Ethynyl estradiol (EE)**	
			35 mg/kg/day		**75 mg/kg/day**		**150 mg/kg/day**			
Study Day	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
1	38.6	2.1	38.0	2.2	37.8	2.4	38.4	2.0	36.8	2.0
3	46.8	2.9	45.9	2.9	42.9	2.9	**35.4****	2.3	44.4	1.9
Cumulative BW gain (1-3)	8.2	1.2	8.0	1.1	**5.1***	1.1	**-3.0****	2.9	7.6	0.8

SD: Standard Deviation
* Significantly different from controls at $p \leq 0.05$
** Significantly different from controls at $p \leq 0.01$

As expected vaginal opening did not occur in any animal (in any group) by the end of the treatment period.

The test was considered acceptable as individual uterine weights in the control group met the acceptance criteria defined in the US EPA Guideline.

Ethynyl estradiol, the reference positive control, administered daily by oral gavage for 3 days at 1 µg/kg/day induced an increase in absolute uterine weights (wet and blotted); ($p \leq 0.01$) and responded as expected.

Table 6.8-26. Terminal body weights and uterine weights after treatment with ethynyl estradiol

	Vehicle		**Ethynyl estradiol 0.001 mg/kg/day**	
Parameter	mean	SD	mean	SD
Terminal Body Weight (g)	51.5	3.0	48.7	1.9
Wet, absolute (mg)	26.4	2.2	**81.9****	23.6
Wet, relative to TBW (%)[$]	0.0514	0.0053	0.1694	0.0524
Blotted, absolute (mg)	23.4	1.8	**70.7****	16.6
Blotted, relative to TBW (%)[$]	0.0455	0.0045	0.1459	0.0364

N=6
SD = Standard Deviation
TBW = terminal body weight

Parameter	Vehicle mean	Vehicle SD	Ethynyl estradiol 0.001 mg/kg/day mean	Ethynyl estradiol 0.001 mg/kg/day SD

* Significantly different from controls at p≤0.05
**Significantly different from controls at p≤0.01
$ No statistical analysis was performed on relative organ weight to terminal body weight ratio.

Tebuconazole did not increase uterine weight up to the highest dose tested (150 mg/kg bw/day). At 150 mg/kg bw/day, the mean terminal body weight was reduced by 20 % when compared to the controls (p≤0.01). The mean uterine weight was slightly decreased when compared to the control group. This effect was associated with a reduced body weight gain and the ratio of absolute uterine weight relative to terminal body weight was comparable to the controls. This unspecific effect was considered to be due to the systemic toxicity of tebuconazole, since animals at this dose level had a lower body weight gain and terminal body weight, when compared to controls, and the relative weight was comparable to that of the controls. At 75 and 35 mg/kg bw/day, the mean terminal body weight was slightly reduced by 7 and 5 % respectively when compared to the controls (not statistically significant).

Table 6.8-27. Terminal body weights and uterine weights after treatment with tebuconazole

Parameter	Vehicle mean	Vehicle SD	Tebuconazole 35 mg/kg/day mean	Tebuconazole 35 mg/kg/day SD	Tebuconazole 75 mg/kg/day mean	Tebuconazole 75 mg/kg/day SD	Tebuconazole 150 mg/kg/day mean	Tebuconazole 150 mg/kg/day SD
Terminal Body Weight (g)	51.5	3.0	48.7	2.7	47.9	2.9	**41.3****	2.8
Wet uterine weight, absolute (mg)	26.4	2.2	24.0#	1.8#	24.9#	3.6	20.5	1.3
Wet uterine weight, relative to TBW (%)$	0.0514	0.0053	0.0487#[1]	0.0045#[1]	0.0519	0.0066	0.0497	0.0037
Blotted uterine weight, absolute (mg)	23.4	1.8	21.1	2.3	22.4	3.0	18.4	0.9
Blotted uterine weight, relative to TBW (%)$	0.0455	0.0045	0.0433	0.0039	0.0469	0.0056	0.0446	0.0027

N=6 (#: N=5; #[1]: wet uterine weight of animal R2F1126 was excluded as aberrant)
SD = Standard Deviation; TBW = Terminal body weight
$ No statistical analysis was performed on relative organ weight to terminal body weight ratio.

Conclusion
Administration of up to 150 mg/kg bw/day tebuconazole for 3 days to immature Sprague-Dawley female rats did not increase uterine weights (wet and blotted). No evidence of an estrogenic potential was detected under these conditions.

Hershberger bioassay

Previous evaluation	None – submitted for the purpose of renewal (study owned by Bayer Task force)

Study ID	B.6.8.3.1.4/22
Study title	Tebuconazole - Evaluation in the Hershberger bioassay
Matrix ID	34
Test substance	Tebuconazole
Purity (%) **Batch no.**	97.5 K689052
Vehicles	Corn oil for TP, 0.5 % Methylcellulose 400 for FT, 0.5 % Methylcellulose 400 and 0.4 % Tween 80 for tebuconazole.
Positive controls	Testosterone propionate (TP) Flutamide (FT)
GLP	Yes
Guideline	OECD 441 (2009)
Deviation	None.

Species/ Strain/ Sex/ Group	Rat Crl:CD (SD) Sprague Dawley Male, castrated. 6/group
Administration	Oral, gavage for vehicle, tebuconazole and FT; TP by subcutaneous injection. 10 consecutive days
Dose (mg/kg bw/day	Screen for androgenic activity: Tebuconazole at 0, 75, 150 (without TP); TP at 0.4 (s.c. injection, positive control). Screen for anti-androgenic activity: Tebuconazole at 35, 75, 150 (with TP at 0.4 by s.c. injection); FT: 3 with TP at 0.4 (s.c. injection, positive control).
Acceptable	Acceptable as part of a weight-of-evidence approach.
Result	Tebuconazole did not show androgenic or anti-androgenic effects.

Methods
This study was conducted according to the OECD guideline 441 (2009) and US-EPA, OPPTS Series 890, Test Guideline N°890.1400 (2009). The objective of this study was to evaluate in a short-term screening test (the Hershberger Bioassay) the potential of tebuconazole for androgen agonist/antagonist and 5α-reductase inhibition properties. This was done by evaluating the weights of the five androgen dependent tissues (the ventral prostate, seminal vesicle (plus fluids and coagulating glands), levator ani-bulbocavernosus (LABC) muscle, paired Cowper's glands and the glans penis) in castrated Sprague-Dawley male rats following exposure to tebuconazole using the oral route of administration (gavage).

To screen for androgenic activity, groups of 6 rats were administered tebuconazole in aqueous formulation of 0.5% methylcellulose 400 + 0.4% Tween 80 by gavage for 10 days at dose levels of 0, 75 and 150 mg/kg/day. Another group received the vehicle by oral gavage and testosterone propionate (TP) by subcutaneous injection at 0.4 mg/kg bw/day (positive control).

To screen for anti-androgenic activity and 5α-reductase inhibition, rats received a daily dose of a potent reference androgen (TP) by subcutaneous injection at 0.4 mg/kg bw/day, as well as a daily oral gavage dose of tebuconazole at dose levels of 0, 35, 75 and 150 mg/kg/day for 10 consecutive days. Another group received TP by subcutaneous injection and flutamide (FT) by gavage at 3 mg/kg bw/day (positive control). All animals were observed for mortality and clinical signs daily; body weights were recorded daily.

At scheduled sacrifice, approximately 24 hours after the last dose, animals were observed for preputial separation and the five androgen dependent tissues were weighed.

Results
There was no mortality or treatment-related clinical signs observed throughout the course of the study. Tebuconazole dosed at 150 mg/kg bw/day (without TP) induced a body weight loss of 1.25 g on Day 2 compared to a body weight gain of 5.37 g in the controls. Overall, the mean absolute body weight gain between Day 1 and 10 was reduced by 20 % ($p \leq 0.05$), when compared to the concurrent controls. Tebuconazole dosed at 150 mg/kg bw/day (with TP) induced a body weight loss of 0.35 g on Day 3 compared to a body weight gain of 10.20 g in the controls ($p \leq 0.01$). Overall, the mean absolute body weight gain between Day 1 and 10 was comparable to the controls (-5 % not statistically significant). At 35 and 75 mg/kg bw/day tebuconazole, with or without TP co-treatment, mean body weight parameters were unaffected throughout the treatment period. On the day of necropsy, the prepuce was separated from the glans penis in all animals.

The organs weights were considered acceptable. TP, the androgenic reference positive control, induced an increase in absolute organ weights ($p \leq 0.01$) and responded as expected (Table 6.8-22). When administered concurrently with TP, FT, the anti-androgenic reference positive control, induced a decrease in absolute organ weights ($p \leq 0.01$) and responded as expected (Table 6.8-23).
Androgenic activity
There was no treatment-related effect on terminal body weight or tissue weights at either dose level of tebuconazole (75 and 150 mg/kg bw/day) (Table 6.8-22). This conclusion was supported by both statistical approaches, i.e. analysis of variance or covariance analysis using the terminal body weight as co-variable.

Table 6.8-28. Organ weights at terminal sacrifice after application of tebuconazole – androgenic activity

	Mean ± standard deviation of absolute organ weight at terminal sacrifice (g)

	(% change versus controls)					
	TBW	**Ventral prostate**	**Seminal vesicles**	**LABC**	**Cowper's glands**	**Glans penis**
Control	360.5 ± 20.4	0.0312 ± 0.0096	0.0449 ± 0.0143	0.2177 ± 0.0728	0.0069 ± 0.0025	0.0503 ± 0.0046
Tebuconazole 75 mg/kg/day	365.8 ± 30.4 (+1%)	0.0232 ±0.0055 (-26%)	0.0471 ± 0.0098 (+5%)	0.2101 ± 0.0571 (-3%)	0.0059 ±0.0022 (-14%)	0.0476 ±0.0059 (-5%)
Tebuconazole 150 mg/kg/day	348.7 ± 19.3 (-3%)	0.0207 ± 0.0057 (-34%)	0.0433 ± 0.0113 (-4%)	0.2107 ± 0.0448 (-3%)	0.0070 ± 0.0016 (+1%)	0.0503 ± 0.0056 (NC)
Testosterone propionate 0.4 mg/kg/day (positive control)	373.0 ± 29.6 (+3%)	0.2189** ± 0.0539 (+602%)	0.4904** ± 0.1721 (+992%)	0.2666** ± 0.0883 (+160%)	0.0379** ± 0.0098 (+449%)	0.0828** ± 0.0076 (+65%)

TBW: terminal body weight; LABC: levator-ani bulbocavernosus muscle
NC: no change
**: p≤0.01

Antagonistic effects
There was no treatment-related effect on terminal body weight or tissue weights at all dose levels of tebuconazole (35, 75 and 150 mg/kw bw/day) (Table 6.8-23). This conclusion is supported by both statistical approaches, i.e. analysis of variance or covariance analysis using the terminal body weight as co-variable

Table 6.8-29. Organ weights at terminal sacrifice after application of tebuconazole – antagonistic effects

	Mean ± standard deviation of absolute organ weight at terminal sacrifice (g) (% change versus controls)					
	TBW	**Ventral prostate**	**Seminal vesicles**	**LABC**	**Cowper's glands**	**Glans penis**
Control TP	373.0 ± 29.6	0.2189 ± 0.0539	0.4904 ± 0.1721	0.5666 ± 0.0883	0.0379 ± 0.0098	0.0828 ± 0.0076
Tebuconazole 35 mg/kg/day	379.4 ± 21.5 (+2%)	0.2012 ± 0.0206 (-8%)	0.5229 ± 0.0953 (+7%)	0.6251 ± 0.0725 (+10%)	0.0309 ± 0.0064 (+3%)	0.0878 ± 0.0121 (+6%)
Tebuconazole 75 mg/kg/day	380.7 ± 19.9 (+2%)	0.2040 ± 0.0206 (-7%)	0.4993 ± 0.0926 (+2%)	0.6440 ± 0.0429 (+14%)	0.0309 ± 0.0112 (-18%)	0.0837 ± 0.0077 (+1%)
Tebuconazole 150 mg/kg/day	371.6 ± 15.8 (NC)	0.1930 ± 0.0225 (-12%)	0.4472 ± 0.1566 (-9%)	0.5721 ± 0.0405 (+1%)	0.0340 ± 0.0106 (-10%)	0.0826 ± 0.0117 (NC)
Flutamide 3 mg/kg/day (positive control)	379.6 ± 32.2 (+2%)	0.0683** ± 0.0192 (-69%)	0.1413** ± 0.0321 (-71%)	0.3491** ± 0.0824 (-38%)	0.0183** ± 0.0057 (-52%)	0.0642** ± 0.0073 (-22%)

TBW: terminal body weight; LABC: levator-ani bulbocavernosus muscle
NC: no change
**: p≤0.01

Conclusion
Administration of up to 150 mg/kg bw/day of tebuconazole for 10 days to castrated Sprague-Dawley male rats had no effect on the five androgen dependent tissues weights. Therefore, tebuconazole was not considered to exhibit any androgenic or anti-androgenic effects under the conditions of this study.

B.6.9. REFERENCES RELIED ON

Literature search

A literature search for tebuconazole and its associated metabolites and impurities has been provided by both Bayer TF and EU Tebuconazole Task Force. These were performed in line with the EFSA guidance (2011) on the submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009. Further detail about each applicants search can be found below.

In addition, RMS-DK conducted a search in open literature on 24 June 2020 to ensure inclusion of all relevant studies. The search string used was *(tebuconazole OR "107534-96-3") AND (rats OR mice OR toxicity OR toxi* OR human OR endocri*)* resulting in 327 publications. Two screening steps were applied to identify relevant studies:
Step 1) Based on title, and if necessary abstract, studies of relevance were identified and categorized. In Step 2) studies were reviewed and 9 *in vivo* studies were found relevant and included in the EDGD table Appendix E. Summary of effects and reliability scores using the Klimisch score system (Klimisch et al. 1997) can be found in Vol 3 B.6. *In vitro* studies were summarised in Vol 3 B.6 and all *in vitro* data entered in Appendix E. *In vitro* study results were compiled for LoE separately from the EDGD functions, as the format of the EDGD table does not suit and support handling of *in vitro* data from the open literature.

In addition, The US EPA ToxCast database, the US EPA Chemistry Dashboard and the US EPA Endocrine Disruption Screening Program for the 21st Century (EDSP21) database of high throughput (in vitro) screening assays were searched for relevant data on Tebuconazole.

Data were gathered in the Excel template provided as Appendix E to the ECHA/EFSA guidance for the identification of endocrine disruptors (2018). According to this template each study was given a unique identification number (Study ID Matrix) that is important for its identification in the data-matrix and Lines of Evidence (LoE) spreadsheets of the Excel.

Bayer Task Force
A literature search for tebuconazole chemical active, synonyms and metabolites, was conducted by Bayer TF for the period 2007 – 2016. By interrogating a wide range of databases (Toxcenter, BIOSIS, Agricola, PQSciTech, MedLine, EsBioBase, EMBASE, CABA, PASCAL, Chemical abstracts. DRUGU, IPA, Registry, SciSearch, FSTA) a total of 3294 publications were identified for further review. 2577 were excluded following rapid assessment for relevance and 715 references were selected for detailed review. 62 publications were identified as potentially relevant to the risk assessment of tebuconazole, with 28 being related to effects on health. The UK-RMS agrees that the criteria for relevance with which decisions to select studies in the dossier were made is suitable. Summary evaluations of potentially relevant publications were submitted to the UK-RMS. These publications have been discussed in this document within the relevant data point.

The literature search for the four common triazole derived metabolites (1,2,4-triazole, triazole acetic acid, triazole alanine and triazole lactic acid) was performed for, and owned by, the Triazole Derivatives Metabolite Group (TDMG), The search was initially conducted in January 2014 (covering 2003 – 2014) and has been updated in June 2015, November 2015, January 2016 and August 2016. This literature search has been previously submitted to support the review of TDMG parent triazole active substances in various EU Member States and has not been repeated in detail here. An overview of the Toxicology results is summarised in the table below:

Data requirement(s) captured in the search	Jan 2014	June 2015	Nov 2015	Jan 2016	Aug 2016
Total number of *summary records* retrieved after *all** searches of peer-reviewed literature (excluding duplicates)	791	219	98	17	66
Number of *summary records* excluded from the search results after rapid assessment for relevance	791	219	98	13	66
Total number of *full-text* documents assessed in detail*	0	0	0	4	0
Number of *studies* excluded from further consideration after detailed assessment for relevance	0	0	0	4	0
Number of *studies* not excluded for relevance after detailed assessment (i.e. relevant studies and studies of unclear relevance)	0	0	0	0	0

*both from bibliographic databases and other sources of peer-reviewed literature

The table below gives an overview of the 28 potentially relevant toxicology publications identified by Bayer TF. The last column provide the location of the assessment and the evaluation of the UK RMS.

Table 6.9-1. All relevant studies and studies of unclear relevance after detailed assessment of full-text documents for relevance

SANCO Data Point [KCA and/or KCP]	Author	Year	Title	Source	Comments on relevance of study by companies according to literature review and MCA5 summaries	UK RMS opinion on relevance
KCA 6.1. Absorption, distribution, metabolism and excretion in mammals	Jonsdottir Svava .acte.Osk; Reffstrup Trine Klein; Petersen Annette; Nielsen Elsa	2016	Physiologically Based Toxicokinetic Models of Tebuconazole and Application in Human Risk Assessment.	Chemical research in toxicology, (2016 May 16) Vol. 29, No. 5, pp. 715-34.	Not relevant for the following reasons: -No data requirement according to Regulation 283/2013. -No new data were generated. The study deals the development of PBTK models for tebuconazole, based primarily on data gained in the regulatory studies.	Publication not summarised in RAR as it does not provide any new data.
KCA 6.1. Absorption, distribution, metabolism and excretion in mammals	Mercadante R; Polledri E; Scurati S; Moretto A; Fustinoni S	2014	Identification and quantification of metabolites of the fungicide tebuconazole in human urine.	Chemical research in toxicology, (2014 Nov 17) Vol. 27, No. 11, pp. 1943-9.	Potentially relevant	RMS evaluation included in the kinetic section.
KCA 6.4.1 *In vitro* genotoxicity studies	Schwarzbacherova, V.; Sivikova, K.; Drazovska, M.;	2015	Evaluation of DNA damage and cytotoxicity induced by	Caryologia (2015), Volume 68, Number 3, pp.	Genotoxicity Not relevant. PPP with 2 active substances was tested.	Publication not summarised in the RAR as it involves a non-standard genotoxicity *in vitro* test and the material tested was a

	Dianovsky, J.		triazole fungicide in cultured bovine lymphocytes.	233-238, 41 refs.		mixture and not tebuconazole alone.
KCA 5.8.2. Supplementary studies on the active substance	Zhou, Jinghua; Zhang, Jianyun; Li, Feixue; Liu, Jing	2016	Triazole fungicide tebuconazole disrupts human placental trophoblast cell functions	Journal of Hazardous Materials (2016), 308, 294-302	Results do not change existing endpoints (NOAEL, ADI, AOEL, MRL, transfer factor, etc. The author himself remarks that further studies are needed to clarify potential risks and it should be kept in mind that the aforementioned data were derived in artificial *in vitro* models with high TEB concentrations. In addition to this, the concentrations which led to effects are too high to be reached under normal exposure conditions in humans. For example, a dose of 100 mg/kg bw leads to an mean unbound tebuconazole plasma concentrations of approximately 1.5 mg/L (0.5 µM), whereas in this study only doses of 10 µM and higher caused some effects.	RMS evaluation included in the reproductive toxicity section.
KCA 5.6.2 Developmental toxicity studies	Di Renzo, F.; Bacchetta, R.; Bizzo, A.; Giavini, E.; Menegola, E.	2013	Is the amphibian X. laevis WEC a good alternative method to rodent WEC teratogenicity assay? The example of the three triazole derivative fungicides Triadimefon, Tebuconazole , Cyproconazole	Reproductive Toxicology (2011), 32(2), 220-226	EFSA guidance point 5.4.1: Reported data do not lead to a more conservative risk assessment; Study is considered to be supplemental information but for the sake of completeness summarized in detail in the Dossier under the given data point. **Not reliable** **Not relevant**	RMS evaluation included in the reproductive toxicity section.
KCA 6.6. Reproductive toxicity	Menegola, Elena; Di Renzo, Francesca; Metruccio, Francesca; Moretto, Angelo (correspondence); Giavini, Erminio	2013	Effects of mixtures of azole fungicides in post implantation rat whole-embryo cultures	Archives of Toxicology, (November 2013) Vol. 87, No. 11, pp. 1989-1997.	Teratology Not relevant. No data requirement according to Regulation 283/2013. Study for mixture effects on whole embryo cultures.	Publication not summarised in RAR as it involves exposure to a mixture rather than tebuconazole alone.
KCA 5.8.2 Supplementary	Zhu, Wentao; Qiu, Jing; Dang, Ziheng;	2007	Stereoselective degradation kinetics of	Chirality (2007), 19(2), 141-147	EFSA guidance point 5.4.1: Reported data do not lead to a more conservative	RMS evaluation included in the kinetic section.

studies on the active substance	Lv, Chunguang; Jia, Guifang; Li, Li; Zhou, Zhiqiang		tebuconazole in rabbits		risk assessment; Study is considered to be supplemental information but for the sake of completeness summarized in detail in the Dossier under the given data point. In the present study, they found that an acute administration of rac-tebuconazole resulted in stereoselective disposition of the (-)-and (+)-enantiomers of tebuconazole. Concentration of the (+)-enantiomer in plasma decreased more rapidly than that of the (-)-enantiomer. This finding was evidenced by the plasma EF values, which increased with time. Plasma protein binding may contribute to these differences. Stereoselective plasma protein binding seems likely in the present study because initial levels of the separate enantiomers were different from each other, and chiral conversion of tebuconazole in plasma may also play a role in these differences. Stereoselective degradation of rac-tebuconazole enantiomers in some tissues was observed. There were several possible factors involved. The first factor was chiral inversion of the two enantiomers in plasma. The second factor was likely stereoselective distribution of (+)- and (-)-tebuconazole in tissues.	
KCA 5.8.2 Supplementary studies on the active substance	Sergent, T.; Dupont, I.; Jassogne, C.; Ribonnet, L.; Van Der Heiden, E.; Scippo, M.; Muller, M.; Mcalister, D.; Pussemier, L.; Larondelle, Y.; Schneider, Y.	2013	CYP1A1 induction and CYP3A4 inhibition by the fungicide imazalil in the human intestinal Caco-2 cells-Comparison with other conazole pesticides	Toxicology Letters (2009), 184(3), 159-168	EFSA guidance point 5.4.1: Reported data do not lead to a more conservative risk assessment; Study is considered to be supplemental information but for the sake of completeness summarized in detail in the Dossier under the given data point. It is concluded that IMA, an imidazole-antifungal pesticide and drug, is a potent inducer of the CYP1A1 enzyme, but also an inhibitor of its activity, as well as a	RMS evaluation included in the carcinogenicity section.

					powerful inhibitor of CYP3A4 activity. In the present study, the effect of four conazole-fungicides, two imidazole-derivatives, i.e. IMA and ketoconazole, and two triazoles, i.e. propiconazole and tebuconazole, on the CYP1A1 activity in human intestinal Caco-2 cells was tested. An inducing effect on the CYP1A1 activity after treatment with the selected azoles was observed, IMA being the most potent inducer. IMA revealed to be a CYP1A1 inducer as potent as B(a)P and TCDD. Tebuconazole, also induced the CYP1A1 activity, but to a much lesser extent than imazalil.	
KCA 5.8.2 Supplementary studies on the active substance	Shen, Z.; Zhu, W.; Liu, D.; Xu, X.; Zhang, P.; Zhou, Z.	2011	Stereoselective degradation of tebuconazole in rat liver microsomes	Chirality, Vol. 24, pp. 67-71	EFSA guidance point 5.4.1: Reported data do not lead to a more conservative risk assessment; Study is considered to be supplemental information but for the sake of completeness summarized in detail in the Dossier under the given data point. Racemic tebuconazole showed stereoselective degradation kinetics in rat liver microsomes which resulted from the competitive interaction effect between the two enantiomers.	RMS evaluation included in the kinetic section.
KCA 5.8.2. Supplementary studies on the active substance	Heusinkveld, Harm J.; Molendijk, Jeffrey; Van Den Berg, Martin; Westerink, Remco H. S.	2013	Azole Fungicides Disturb Intracellular Ca2+ in an Additive Manner in Dopaminergic PC12 Cells	Toxicological Sciences (2013), 134(2), 374-381	Results do not change existing endpoints (NOAEL, ADI, AOEL, MRL, transfer factor, etc.) The present results demonstrate that the azole fungicides imazalil, flusilazole, triadimefon, tebuconazole, and cyproconazole concentration-dependently inhibit depolarization-evoked calcium influx. Fluconazole does not induce an inhibition of depolarization-evoked calcium influx with exposures up to 100 µM. All five compounds induce a (near) complete inhibition at the highest concentrations, indicative of a nonspecific	RMS evaluation included in the neurotoxicity section.

					inhibition of VGCCs. IC50 values range from 5 µM (flusilazole) to 65 µM (cyproconazole), revealing a one order of magnitude difference in potency. Exposure of cells to binary IC20 or quaternary IC10 mixtures provides clear indications for additivity with respect to inhibition of depolarization-evoked calcium influx. The results of the oxidative stress assay indicate only an increase in oxidative stress for exposure to imazalil and flusilazole (100 µM). The other four fungicides, i.e. also tebuconazole did not induce an effect on oxidative stress.	
KCA 5.8.2. Supplementary studies on the active substance	Tamura, Kei; Inoue, Kaoru; Takahashi, Miwa; Matsuo, Saori; Irie, Kaoru; Kodama, Yukio; Gamo, Toshie; Ozawa, Shogo; Yoshida, Midori	2015	Involvement of constitutive androstane receptor in liver hypertrophy and liver tumor development induced by triazole fungicides	Food and Chemical Toxicology (2015), 78, 86-95	Results do not change existing endpoints (NOAEL, ADI, AOEL, MRL, transfer factor, etc.) Repeated dose tox, carcinogenicity (ED) Not relevant for the following reasons: -No clarification of MoA for Tebuconazole -No test guideline-compliance stated. MoA study on development of liver hypertrophy and of liver tumours. Not relevant for risk assessment. The authors conclude that Tebuconazole liver hypertrophy upon very high exposure is independent from CAR-mediated liver tumor development in rodents and may therefore be not relevant to humans. For Tebuconazole-induced liver hypertrophy they hypothize a possible involvement of PXR.	RMS evaluation included in the carcinogenicity section.
KCA 5.8.2. Supplementary studies on the active substance	Tamura, Kei; Inoue, Kaoru; Takahashi, Miwa; Matsuo, Saori; Irie, Kaoru; Kodama, Yukio;	2013	Dose-response involvement of constitutive androstane receptor in mouse liver hypertrophy induced by	Toxicology Letters (2013), 221(1), 47-56	Results do not change existing endpoints (NOAEL, ADI, AOEL, MRL, transfer factor, etc.) The authors conclude that the present	RMS evaluation included in the carcinogenicity section.

	Ozawa, Shogo; Nishikawa, Akiyoshi; Yoshida, Midori		triazole fungicides		study demonstrated that all triazoles examined had dose-responsive involvement of CAR in liver hypertrophy. Cypro or Flu induced mainly CAR-mediated liver hypertrophy, but CAR was only slightly involved in Teb-induced hypertrophy. The involvement of non-CAR routes, including PXR, was also evaluated for these triazoles, while PB produced hypertrophy that had a pattern indicating complete dependence on CAR.	
KCA 5.8.3 Endocrine disrupting properties	Laws, S.; Yavanhxay, S.; Cooper, R. L.; Eldridge, J. C.	2006	Nature of the binding interaction for 50 structurally diverse chemicals with rat oestrogen receptors	Toxicological Sciences (2006), Vol. 94, Iss. 1, pp. 46-56	EFSA guidance point 5.4.1: Reported data do not lead to a more conservative risk assessment; Study is considered to be supplemental information but for the sake of completeness summarized in detail in the Dossier under the given data point. Tebuconazole is inactive in the oestrogen receptor binding study.	RMS evaluation included in the ED section.
KCA 5.8.3 Endocrine disrupting properties	Rieke, S.; Koehn, S.; Hirsch-Ernst, K.; Pfeil, R.; Kneuer, C.; Marx-Stoelting, P.	2014	Combination effects of (tri)azole fungicides on hormone production and xenobiotic metabolism in a human placental cell line	International Journal of Environmental Research and Public Health (2014), 11(9), 9660-9679	EFSA guidance point 5.4.1: Reported data do not lead to a more conservative risk assessment; Study is considered to be supplemental information but for the sake of completeness summarized in detail in the Dossier under the given data point. Not included for the following reason: -study data addressed already in the separate statement on Endocrine Disruption by Philip Bentley (2017; see KCA 5.8.3/01), supplementary information. (*in vitro* assay for anti-androgenic effects, including tebuconazole) **Not reliable** **Relevant with restrictions** In Jegg-3 cells tebuconazole decreased progesterone synthesis at 15 µM and increased Cyp1a1 expression at 3 µM. 30	RMS evaluation included in the ED section.

					µM tebuconazole-induced Cyp1a1 expression can be antagonized by 10 µM CH223191 AhR-antagonist.	
KCA 5.8.3. Endocrine disrupting properties	Dreisig, Karin; Taxvig, Camilla; Kjaerstad, Mia Birkhoj; Nellemann, Christine; Hass, Ulla; Vinggaard, Anne Marie, Dr. (Correspondence)	2013	Predictive value of cell assays for developmental toxicity and embryotoxicity of conazole fungicides.	Altex, (2013) Vol. 30, No. 3, pp. 319-330. Refs: 99 ISSN: 1868-596X; E-ISSN: 1868-8551 CODEN: ALTEEK	Results do not change existing endpoints (NOAEL, ADI, AOEL, MRL, transfer factor, etc.) **Reliable with restrictions (Klimisch score 2)** **Not relevant** Although the reported effective tebuconazole concentrations in the EST are not relevant for the *in vivo* situation, the results show that tebuconazole has the weakest *in vitro* embryotoxic potential in relation to the other tested azole compounds (ketoconazole > epoxiconazole ≈ prochloraz > propiconazole ≈ tebuconazole). Moreover, the authors show based on publically available data from their own in-house experiments or others' studies that this order is fairly similar for the *in vivo* situation with tebuconazole being also the least embryotoxic compound *in vivo* of the 5 tested azoles (ketoconazole > epoxiconazole > prochloraz > propiconazole ≈ tebuconazole).	RMS evaluation included in the reproductive toxicity section.
KCA 5.8.3. Endocrine disrupting properties	Hass, Ulla; Boberg, Julie; Christiansen, Sofie; Jacobsen, Pernille Rosenskjold; Vinggaard, Anne Marie; Taxvig, Camilla; Poulsen, Mette Erecius; Herrmann, Susan Strange; Jensen, Bodil Hamborg; Petersen, Annette; Clemmensen, Line	2012	Adverse effects on sexual development in rat offspring after low dose exposure to a mixture of endocrine disrupting pesticides	Reproductive Toxicology (2012), 34(2), 261-274	Results do not change existing endpoints (NOAEL, ADI, AOEL, MRL, transfer factor, etc.) Maternal NOAEL = 50 mg/kg bw/day Developmental LOAELfemales = 12.5 mg/kg bw/day, increased AGDI Developmental LOAELmales = 50 mg/kg bw/day, increased nipple retention **Not reliable** **Relevant with restrictions**	RMS evaluation included in the reproductive toxicity section.

	Harder; Axelstad, Marta					
KCA 5.8.3. Endocrine disrupting properties	Jacobsen, Pernille Rosenskjold; Axelstad, Marta; Boberg, Julie; Isling, Louise Krag; Christiansen, Sofie; Mandrup, Karen Riiber; Berthelsen, Line Olrik; Vinggaard, Anne Marie; Hass, Ulla	2012	Persistent developmental toxicity in rat offspring after low dose exposure to a mixture of endocrine disrupting pesticides	Reproductive Toxicology (2012), 34(2), 237-250	EFSA guidance point 5.4.1: Reported data do not lead to a more conservative risk assessment; Study is considered to be supplemental information but for the sake of completeness summarized in detail in the Dossier under the given data point. In general, no statistically significant effects were observed for tebuconazole on hormone levels, semen quality, organ weights/histology, onset of puberty and behaviour/learning up to and including the highest tested dose of 50 mg/kg bw/day. The only exceptions were the increased liver weight in PD 16 male offspring at the high dose level (but no effect in adult offspring and no accompanying histological findings) and the increased total motor activity in adult female offspring and the increased swim length and latency in male offspring, both only in the low dose group. Adverse effects were observed in young and adult male offspring from the group exposed to the highest dose of the pesticide mixture. These included reduced prostate and epididymis weights, increased testes weights, altered prostate histopathology, increased density of mammary glands, reduced sperm counts, and decreased spatial learning. **Not reliable** **Relevant with restrictions**	RMS evaluation included in the reproductive toxicity section.
KCA 5.8.3. Endocrine disrupting properties	Kjaerstad, M. B.; Taxvig, C.; Andersen, H. R.; Nellemann, C.	2010a	Mixture effects of endocrine disrupting compounds *in vitro*	International Journal of Andrology (2010), 33(2), 425-433	EFSA guidance point 5.4.1: Reported data do not lead to a more conservative risk assessment; Study is considered to be supplemental information but for the sake of completeness summarized in detail in the Dossier under the given data point.	RMS evaluation included in the ED section.

					Reliable with restrictions **Relevant with restrictions**	
KCA 5.8.3. Endocrine disrupting properties	Kjaerstad, Mia B.; Taxvig, Camilla; Nellemann, Christine; Vinggaard, Anne Marie; Andersen, Helle R.	2010b	Endocrine disrupting effects *in vitro* of conazole antifungals used as pesticides and pharmaceuticals	Reproductive Toxicology (2010), 30(4), 573-582	Results do not change existing endpoints (NOAEL, ADI, AOEL, MRL, transfer factor, etc.) Not included for the following reason: -study data addressed already in the separate statement on Endocrine Disruption by Philip Bentley (2017; see KCA 5.8.3/01), supplementary information. (Different *in vitro* test systems applied for evaluation of effects on sexual hormone synthesis with positive (anti-androgen, anti-estrogenic) results, due to effect on steroidogenesis) **Not reliable** **Not relevant**	RMS evaluation included in the ED section.
KCA 5.8.3. Endocrine disrupting properties	Orton, Frances; Rosivatz, Erika; Scholze, Martin; Kortenkamp, Andreas	2011	Widely used pesticides with previously unknown endocrine activity revealed as *in vitro* antiandrogens	Environmental Health Perspectives (2011), 119(6), 794-800	Results do not change existing endpoints (NOAEL, ADI, AOEL, MRL, transfer factor, etc.) Not included for the following reason: -study data addressed already in the separate statement on Endocrine Disruption by Philip Bentley (2017; see KCA 5.8.3/01), supplementary information. (*in vitro* assay for anti-androgenic effects, including tebuconazole)	Publication not summarised in RAR as it does not provide primary data.
KCA 5.8.3. Endocrine disrupting properties	Orton, Frances; Rosivatz, Erika; Scholze, Martin; Kortenkamp, Andreas	2012	Competitive androgen receptor antagonism as a factor determining the predictability of cumulative antiandrogenic effects of widely used	Environmental Health Perspectives (2012), 120(11), 1578-1584	Results do not change existing endpoints (NOAEL, ADI, AOEL, MRL, transfer factor, etc.)	Publication not summarised in RAR as it does not provide primary data.

			pesticides			
KCA 5.8.3. Endocrine disrupting properties	Overgaard, Agnete; Holst, Klaus; Mandrup, Karen R.; Boberg, Julie; Christiansen, Sofie; Jacobsen, Pernille R.; Hass, Ulla; Mikkelsen, Jens D.	2013	The effect of perinatal exposure to ethinyl oestradiol or a mixture of endocrine disrupting pesticides on kisspeptin neurons in the rat hypothalamus	NeuroToxicology (2013), 37, 154-162	Results do not change existing endpoints (NOAEL, ADI, AOEL, MRL, transfer factor, etc.) In summary, tebuconazole had no effect on preputial separation (males), vaginal opening (females) or Kisspeptin mRNA expression in doses up to an including the highest tested dose of 50 mg/kg bw/day. Similarly, neither perinatal EE2 nor exposure to the other pesticides did affect Kiss1 mRNA expression. EE2 had minor effects on puberty onset. **Not reliable** **Restricted relevance**	RMS evaluation included in the reproductive toxicity section.
KCA 5.8.3. Endocrine disrupting properties	Taxvig, C.; Vinggaard, A. M.; Hass, U.; Axelstad, M.; Metzdorff, S.; Nellemann, C.	2008	Endocrine-disrupting properties *in vivo* of widely used azole fungicides	International Journal of Andrology (2008), 31(2), 170-177	Results do not change existing endpoints (NOAEL, ADI, AOEL, MRL, transfer factor, etc.) Not included for the following reason: -study data addressed already in the separate statement on Endocrine Disruption by Philip Bentley (2017; see KCA 5.8.3/01), supplementary information. (Hershberger assay and teratogenicity study with positive results for tebuconazole) **Not reliable** **Relevant with restrictions**	RMS evaluation included in the reproductive toxicity section.
KCA 5.8.3. Endocrine disrupting properties	Taxvig, Camilla; Hass, Ulla; Axelstad, Marta; Dalgaard, Majken; Boberg, Julie; Andeasen, Helle Raun; Vinggaard, Anne Marie	2007	Endocrine-Disrupting Activities In Vivo of the Fungicides Tebuconazole and Epoxiconazole	Toxicological Sciences (2007), 100(2), 464-473	Results do not change existing endpoints (NOAEL, ADI, AOEL, MRL, transfer factor, etc.) Not relevant for the following reasons: No guideline stated, study not guideline-compliant (rather teratology and segment 3 study combined, only 2 dose groups per test substance, only approx. 10 animals	RMS evaluation included in the reproductive toxicity section.

					per dose), thus no reliable data **Reliable with restrictions** **Relevant with restrictions**	
KCA 5.8.3. Endocrine disrupting properties	Kugathas, Subramaniam; Audouze, Karine; Ermler, Sibylle; Orton, Frances; Rosivatz, Erika; Scholze, Martin; Kortenkamp, Andreas	2016	Effects of common pesticides on prostaglandin D2 (PGD2) inhibition in SC5 mouse sertoli cells, evidence of binding at the cox-2 active site, and implications for endocrine disruption.	Environmental Health Perspectives, (April 2016) Vol. 124, No. 4, pp. 452-459.	Not relevant for the following reasons: -No valid guideline stated -test items are not appropriately characterized in the publication. (Study with 24 pesticides on mouse sertoli cells. Tebuconazole suppresses PGD2 production (so do 14 other substances tested). This effect is supposed connected to anti-androgen effects)	Publication not summarised in RAR as it involves an non-standard test.
KCA 5.8.3. Endocrine disrupting properties	Marx-Stoelting, P. (correspondence); Niemann, L.; Ritz, V.; Ulbrich, B.; Gall, A.; Hirsch-Ernst, K.I.; Pfeil, R.; Solecki, R.	2014	Assessment of three approaches for regulatory decision making on pesticides with endocrine disrupting properties.	Regulatory Toxicology and Pharmacology, (December 01, 2014) Vol. 70, No. 3, pp. 590-604.	Not relevant for the following reason: -Workshop exercise by regulatory people for three theoretical approaches to assess the potential of endocrine disruption, with tebuconazole selected as one pesticide among others. -no new data generated.	Publication not summarised in RAR as it does not provide primary data.
KCA 5.8.3. Endocrine disrupting properties	Roelofs, Maarke J. E.; Temming, A. Roberto; Piersma, Aldert H.; van den Berg, Martin; van Duursen, Majorie B. M.	2014	Conazole fungicides inhibit Leydig cell testosterone secretion and androgen receptor activation *in vitro*	Toxicology Reports, (2014) Vol. 1, pp. 271-283.	Not included for the following reason: -study data addressed already in the separate statement on Endocrine Disruption by Philip Bentley (2017; see KCA 5.8.3/01), supplementary information. (*in vitro* assay for anti-androgenic effects, including tebuconazole as one test item).	Publication not summarised in RAR as it does not provide primary data.

EU Tebuconazole Task Force

A literature search for tebuconazole chemical active, its major metabolites, and plant protection products containing tebuconazole was conducted by EU Tebuconazole Task Force for the period 2006 – 2016. A total of 12280 reference citations were identified after interrogating multiple databases (RTECS, MSDS, REAXYSFILE, DETHERM, HSDB). 1523 summary records were retrieved considering keywords, and 533 of these were included following rapid relevance assessment. 137 abstracts were assessed and 74 of these full text documents were evaluated in detail. Nine references were identified as being relevant or of unclear relevance. These overlaps with the 28 publications identified as potentially relevant by the Bayer TF. The RMS note that the relevance criteria used by the EU Tebuconazole Task Force were more restrictive.

Table 6.9-2. Results of the study selection process

Data requirement(s) captured in the search	**Number**
Total number of *summary records* retrieved after all* searches of peer-reviewed literature (excluding duplicates)	12280
Total number of *summary records* retrieved after all* searches of peer-reviewed literature (excluding duplicates) considering keywords	1523
Number of *summary records* not excluded from search results after rapid assessment for relevance	533
Total number of *abstracts* assessed	137
Total number of *full-text* documents assessed in detail	74
Number of *studies* excluded from further consideration after detailed assessment for relevance	64
Number of *studies* not excluded for relevance after detailed assessment (i.e. relevant studies and studies of unclear relevance)	9