Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

to the Opinion proposing harmonised classification and labelling at Community level of

Metazachlor

ECHA/RAC/ CLH-O-0000001586-69-01/A2

Adopted
8 March 2011

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPSAL ON METAZACHLOR

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

[ECHA has compiled the comments received via internet that refer to several hazard classes and entered them under each of the relevant categories/headings as comprehensive as possible. Please note that some of the comments might occur under several headings when splitting the given information is not reasonable.]

Substance name: METAZACHLOR
CAS number: 67129-08-2
EC number: 266-583-0

General comments

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
08/04/2010	Portugal / Maria do Carmo Palma / MSCA	Considering the present proposal, we agree to establish an harmonised classification & labelling for Metazachlor. The proposed Classification and Labelling fulfills the criteria established both in CLP Regulation and 67/548/EEC Directive (human health and environment). Therefore, we support this proposal. Nevertheless, there seems to be a minor inconsistency in the conclusion written in page 64 that should be corrected. Therefore we suggest the replacement of "Metazachlor and its degradants exhibited limited acute toxicity to fish and invertebrates compared to other trophic levels, with the lowest 48-h LC50 of 8.5 mg a.s. /l for fish", by "Metazachlor and its degradants exhibited limited acute toxicity to fish and invertebrates compared to other trophic levels, with the lowest 96-h LC50 of 8.5 mg/l for fish".	Thank you for agreeing to our proposal Thank you for spotting this error – we have made the correction.	Noted Text in BD has been changed.

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Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
14/04/2010	France / Antony Fastier / AFFSA	We agree with the classification proposal : R43: May cause sensitisation by skin contact R40 (Carc. Cat 3): Limited evidence of a carcinogenic effect	Thank you for agreeing with our proposal	Noted.
14/04/2010	Germany / Jan Averbecl / MSCA	The German CA supports to harmonize the classification & labelling for Metazachlor. The data of several carcinogenicity studies were re-evaluated by BASF and a pathologist expert group. The results about this work should be integrated into this document e.g. in form of tables with the re-evaluated data. The incidences re-evaluated by several pathologist expert group as well as adequate historical control data are considered essential for a final conclusion. Additional information on the mode of action and human relevance for formation of the relevant tumours would be very helpful.	Thank you for agreeing to our proposal Since three separate reviews of the tumour findings were conducted a lot of information is available. We felt that it was clearer if this information was presented separately and therefore decided to dedicate a separate section to it. The information can be found in Appendix 1 to the CLH report.	Noted.
21/04/2010	Belgium / Frederic Denauw / MSCA	CLH proposal UK MSCA Signal word : warning Classification : Carc. 2 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1 H-statements : H351 : Suspected of causing cancer H317 : may cause an allergic skin reaction H400 : very toxic to aquatic life H410 : very toxic to aquatic life with long lasting effects	Thank you for agreeing to our environmental classification proposal.	Noted An M-factor of 100 for chronic toxicity (based on NOEC) has been added to the M-factor for acute toxicity based on LC50. Now, we have two separate M-factors, the value of both are 100.

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		M-factor : 100 (based on 0.001 < LC50 ≤ 0.01 mg/l Overall conclusion and Comments : Based on the results of the aquatic acute toxicity test on the most sensitive species (aquatic plant Lemna spp.7dEC50 = 0.0071 mg/L), the fact that the substance is not readily biodegradable and that the substance shows no potential to bioaccumulate (log Kow = 2.49 <4), it is justified to classify as Aquatic Acute 1 and Aquatic Chronic 1. Based on the classification and labelling criteria in accordance with dir. 67/548/EEC, metazachlor should be labelled as N, R50/53, S60, S61. Application of the translation table of annex VII of the CLP regulation 1272/2008, results in the corresponding classification as Aquatic Acute 1, Aquatic Chronic 1. In view of the proposed classification and the toxicity band between 0.001 and 0.01mg/l, a M-factor of 100 could be assigned. In conclusion : we agree with the proposed environmental classification by the UK MSCA. Some minor comments: p.10 Table 1, IX, 7.16 : "… from ionic species" should be "… form ionic		We do not apply the translation table, but we perform a second evaluation based on CLP criteria. Nevertheless, in the case of metazachlor the two evaluations are in agreement. An additional M-factor of 100 for chronic toxicity (based on NOEC) has been added to the M-factor for acute toxicity based on LC50. Now, we have two separate M-factors, one for acute and for chronic aquatic toxicity, both are 100.

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		species" p. 61 Long term toxicity to aquatic invertebrates CLH report : study 1: the number of the EEC guideline is not mentioned	The guideline for study 1 was EEC XI/681/86. On checking this information, we noticed that the study report date was incorrectly cited as 1991 (we have now changed it to 1990).	Thanks: "from" corrected to "form"
26/04/2010	Germany / Christiane Wiemann / BASF SE and Feinchemie Schwebda GmbH	Comments of both manufacturers of metazachlor (BASF SE and Feinchemie Schwebda GmbH) are mainly focusing on the data set relevant for the assessment of potential evidence of carcinogenic properties. However, other aspects of the report are also covered were considered necessary. It is the manufacturers opinion that the slight incidences of benign kidney adenomas of male mice in one of the two submitted studies is not considered treatment related as they are not dose-dependent, not seen in a second study at even higher dose levels and not related to any indication of kidney structural alterations. The slight increased incidence in benign liver adenomas of female Wistar rats at the highest dose is considered most likely treatment-related but not considered relevant for humans based on a phenobarbitone-like enzyme induction (Constitutive Androstane Receptor activation). It is the manufacturers' opinion that when applying the criteria and considerations of the CLP Regulation 1272/2008 a	We have had numerous discussions with the companies concerned, and these issues were addressed and considered during the drafting of our proposal. Consequently, we feel that these comments have now been submitted to aid RAC discussions. Against this background we do not plan to change our position or significantly amend our CLH report. However, we are happy to answer any questions the rapporteur may have and provide assistance.	Noted, comments have been considered in the draft opinion.

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		classification of metazachlor as "Carc. 2 H351: suspected of causing cancer" is not warranted. The studies conducted do not demonstrate limited evidence (suspected human carcinogen) when applying the given criteria: • The slight increased incidence of liver tumours was observed in one species only, i.e. the rat • These slight increased incidences were observed in one of the two studies only • The slight increase incidence was observed in one sex only, i.e. females • The slight increased incidences in rat liver tumours were seen at high dose only (with evidence of excessive toxicity, i.e. 10% retardation in weight gain) • There is no evidence for malignant neoplasm or progression to malignancy- only a slightly increased benign tumour incidence is under consideration • There is no multi-site response in the rat • There is no mode of action identified with relevance for humans For an assessment on the carcinogenic potential an experienced view on the full picture has to be done. The complexity of the available data however does not make the understanding easy. This is due to the following reasons.		

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		• The two manufacturers provided two complete toxicological data sets during re-registration as a pesticide in the EU. • The tumour incidences under discussion are very small and not consistent within or among each data set (e.g. tumour incidences seen in one sex and one of the two studies per species only). • Diagnostic criteria used in the original studies are not comparable due to time shift in the criteria definitions. • A peer review was conducted of all organs and tissues with potentially relevant tumour incidences by manufacturer's pathologists (BASF pathologists). They applied consistent and state of the art diagnostic criteria, which let in some cases to an evaluation different to the evaluation of the original pathologists. • A Pathology Working Group (PWG) was organized to clarify any discrepancies noticed between the first evaluation of the study pathologist and the peer-review of the BASF pathologists and to come to a final conclusion. • An extended mechanistic data set has been prepared to assess the potential underlying toxicological modes of action. The toxicological data set has been extended after the Annex I inclusion decision and is significantly different from the data set presented in the Draft Assessment Report prepared during EU Re-registration of metazachlor with		

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		regard to: • the histopathological peer-review followed by the PWG assessment on the basis of internationally harmonized state of the art diagnostic criteria resulted in deviating tumour incidences in some cases • additional historical control data that have been provided • mechanistic studies on liver toxicity in the rat • mechanistic studies on thyroid toxicity in the rat • mechanistic studies on urinary bladder and kidney toxicity in the mouse Consequently, the manufacturers provide attached to this document documents on the available data set and provide background information on evaluation criteria and scientific approaches. The documents furthermore provide manufacturers' comments and explanations on aspects which in their opinion are not appropriately covered in the Annex VI report. As the size of the attached documents is extending the 10 MByte limit it will be provided in 4 separate submission as agreed upon with the RAC secretariate. A list of all studies and documents submitted is also attached. In conclusion, the available entire data set	To aid the rapporteur we have listed the data submitted by industry (refer to Annex 3) and included a comment to indicate what action has been taken with this information. It should be noted that some of this information was already included in the original submission. All of the submitted data have been	

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		does not justify classification of metazachlor with R40 (Carc. Cat. 3: limited evidence of carcinogenic effect based on Directive 67/548/EEC; Carc. 2 H351: suspected of causing cancer based on the CLP Regulation 1272/2008).	attached to the IUCLID. In addition, please see the attached Annex 2 which contains a summary of some of these new data submitted in support of a phenobarbitone-like mode of action and a summary of the additional historical control data presented. The studies summarised include: • The effects of Metazachlor on CAR activation: a mechanism for the observed CYP2B induction (Wang, 2010) • Induction of the CYP2B1 promoter by metazachlor-dependant CAR (NR1I3) activation in primary cultures of rat hepatocytes (Neuschafer-Rube and Puschel, 2010) • S-phase response study in Wistar Rats administration in the diet for 3, 7, 14 and 28 days (Buesen et al, 2010) • S-phase response study in CD-1 mice administration in the diet for 7, 28 and 91 days (Buesen et al, 2010) and re-examination of data (Hard GC, 2010) • Historical control data	

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Carcinogenicity

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02/04/2010	US / Henry Wall / Experimental Pathology Labortories, Inc.	The following response is submitted on behalf of the Pathology Working Group that performed an independent assessment of the carcinogenic potential of metazachlor as documented in the Annex VI Report. 1. Section 5.8, paragraph 4 (page 37 of 76): In our view the ECHA comments pertaining to the Pathology Working Group (PWG) findings in Section 5.8, paragraph 4 (page 37), that "it is not appropriate to consider the results conclusive because some lesions may have been missed" reflects a misunderstanding of the PWG process. The primary intent of the PWG is to achieve consensus on diagnoses for which there are differences between original study pathologist and the peer review pathologist and to ensure that the diagnoses are in accordance with current diagnostic standards. The peer review step that precedes the PWG review is a 100% review of all tissue sections for a potential target organ by the reviewing pathologist. The results of the PWG are achieved via the independent blinded review of all tissues sections for which there were differences between the original study pathologist and the peer review pathologist followed by critical discussion of criteria and morphologic	Thank you for these comments. The comment included in our proposal was not meant as a criticism of the PWG process. Our concern was that since the two pathologists had used two different criteria the 'review' was essentially an evaluation and, as such, not all slides with lesions may have been identified (especially as the PWG identified a greater number of follicular cell adenomas than either the study or reviewing pathologist). All available information was taken into consideration during the development of our proposal. However, even considering the results of the PWG alone, we do not feel that the outcome is affected. The effects observed in the liver of rat (Wistar) and the kidney of the mouse (CD-1) are still of concern as detailed in the CLH report.	Comment has been regarded.

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		features of a given tissue when necessary to provide understanding of the basis for the consensus diagnosis. We do not agree that the skepticism "more adenomas may have been identified in all dose groups" is a professionally acceptable characterization of the PWG process applied for the review of the carcinogenic potential of metzachlor. The PWG process is not a new one that exists only because of the present consideration of the proposed harmonized classification of metzachlor. It is a widely used and respected process for achieving final diagnoses for pathological changes in experimental toxicology studies when there are differences between the study pathologist and the peer review pathologists. Formally, this process was adopted by the United States Environmental Protection Agency (USEPA): • Pesticide Registration (PR) Notice 94-5: Requests for Re-considerations of Carcinogenicity Peer Review Decisions Based on Changes in Pathology Diagnoses, August 24, 1994. This process has been in longstanding use by the National Toxicology Program (NTP) of the United States National Institutes of Environmental Health Sciences:		

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		• Boorman GA, Montgomery CA Jr, Eustis SL, Wolfe MJ, McConnell EE, Hardisty JF. 1985. Quality assurance in pathology for rodent carcinogenicity studies. In Handbook of Carcinogen Testing; Milman HA, Weisburger EK, Eds; Noyes Publications, park Ridge, New Jersey, pp 345-357. • Boorman GA, Eustis SI. 1986. The pathology working group as a means for assuring pathology quality in toxicological studies. In Managing Conduct and Data Quality of Toxicology Studies; Conference Proceedings, Raleigh, North Carolina, November 18-20, 1985; Hoover BK, Baldwin JK, Uelner AF, Whitmire CE, Davies CL, Bristol DW, Eds; Princeton Scientific Publishing Co., Inc., Princeton, New Jersey; pp 271-275. This process, as applied in the assessment of the carcinogenic potential of metazachlor, has been endorsed by the Society of Toxicologic Pathology and multiple authors with direct involvement in the practice of toxicologic pathology: • The Society of Toxicologic Pathologists. 1991. Peer review in toxicologic pathology: some recommendations. Toxicol Pathol 19(3): 290-292. • Ward JM, Hardisty JF, Hailey JR, Streett CS. 1995. Peer review in toxicologic pathology. Toxicol Pathol 23(2): 116-234.		

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		• Crissman JW, Goodman DG, Hildebrandt PK, Maronpot RR, Prater DA, Riley JH, Seaman WJ, Thake DC. 2004. Best practices guideline: toxicologic histopathology. Toxicol Pathol 32:126-131. As appropriate for the Pathology Working Group, the panel of pathologists consisted of individuals with extensive experience and in the assessment of carcinogenesis in rodents exposed to xenobiotics. We believe that the PWG findings deserve an objective assessment that is free of unsupported speculation. 2. Section 5.8.1.2. "Kidneys", paragraph 4 (page 45 of 76) With regards to the interpretation of mouse kidney tumors there were two 2-year studies performed in mice, the HRC study and the Rallis study. The PWG confirmed a slight increase in the incidence of kidney tumors in the mid- (700 ppm) and high-dose (2500 ppm) groups in the HRC study. However, the PWG would re-emphasize its conclusion that the kidney tumors in male mice in the HRC study were not considered to be treatment related due to the very low incidence, lack of a dose-response relationship. In the Rallis study no increase in tumors were observed an even higher dose (4000 ppm). The difference	With regards to the kidney tumours, we already took the opinions of the PWG into consideration and do not intend to change our proposal at this point. We are happy to assist the rapporteur in the development of their proposal.	

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		in response in the two studies and the fact that male mice have higher spontaneous kidney tumor rates than females (historical control data below, and Giknis and Clifford, 2005; Maita et al., 1988) supports the PWG conclusion that increases in kidney tumors are unlikely to be associated with exposure to metazachlor. We believe that the agency should not conclude that there is a causal association between exposure to metzachlor and the occurrence of kidney tumors in male mice in the studies that we have evaluated. • Giknis MLA, Clifford CB. 2005. Spontaneous neoplastic lesions in the Crl:CD-1 (ICR) Mouse in control groups from 18 month to 2 year studies. Charles River Laboratories, Wilmington, Massachusetts, 19 pp. • Maita K, Hirano M, Harada T, Mitsumori K, Yoshida A, Takahashi K, Nakashima N, Kitazawa T, Enomoto A, Inui K, Shirasu Y. 1988. Mortality, major cause of morbidity, and spontaneous tumors in CD-1 Mice. Toxicol Pathol 16(3):340-349. HENRY G. WALL, D.V.M., Ph.D. Diplomate, ACVP, ABT Veterinary Pathologist Chairperson, Pathology Working Group Historical control data of kidney tumors		

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		(CD-1/Swiss Albino mice Rallis Study Dates 12/99-06/01; HRC Study Dates 04/80-04/82) Kidney CD-11 mice/Swiss Albino mice Adenoma, renal tubule Male Female Strain Time frame HLS2 9/2989 [0.3% (0%-1.98%)] 1/2980 [0.03% (0%-1.92%)] CD-1 06/78-10/84 Advinus3 11/800 [1.4% (0%-6%)] 0/800 Swiss albino 09/96- 08/04 RITA4 8/1348 [0.6% (0%-4%)] 1/1214 [0.1% (0%-2%)] CD-1 07/93-03/03 Kidney CD-1 mice/Swiss Albino mice Carcinoma, renal tubule Male Female Strain Time frame HLS 8/2989 [0.27% (0%-3.85%)] 0/2980 CD-1 06/78-10/84 Advinus 1/800 [0.13% (0%-2%)] 0/800 Swiss albino 09/96- 08/04 RITA 3/1348 [0.2% (0%-2%)] 0/1214 CD-1 07/93-03/03 Kidney CD-1 mice/Swiss Albino mice Combined incidence of adenoma and carcinoma, renal tubule male Female Strain Time frame Advinus 12/800		

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		[1.53% (0%-8%)] 0 Swiss albino 09/96-08/04 RITA 11/1348 [0.8% (0%-6%)] 1/1214 [0.1% (0%-2%)] CD-1 07/93-03/03 HLS 17/2989 [0.57% (0%-5.83%)] 1/2980 [0.03% (0%-1.92%)] CD-1 06/78-10/84 1The CD-1 mouse is a Swiss derived mouse breed originating from Charles River 2 Formerly Huntingdon Research Center (HRC) 3 Formerly Rallis Research Center (Rallis) 4 Registry of Industrial Toxicology Animal-data (RITA) (Mohr and Morawietz 1993, Deschl et al. 2002, http://www.item.fraunhofer.de/reni/public/rita/index.php)		
05/04/2010	New Zealand / Gordon Hard	Page 45 of 76 I wish to take the opportunity offered by ECHA to comment on the renal tubule tumor findings in chronic studies with metazachlor in mice. Specifically, my comments relate to the assessment in the Annex VI report "Proposal for Harmonized Classification and Labeling": "…. these results suggest a weak carcinogenic response in the kidneys of CD-1 mice …." (page 45 of 76, under "Kidneys" 4th paragraph). I have worked in the area of renal toxicology and carcinogenesis for 40 years, either as a researcher or in a	Thank you for these comments. We have assessed these new data and do not consider that they alter our position with regards the kidney adenomas. The information is summarised in Annex 2: S-phase response study in CD-1 mice administration in the diet for 7, 28 and 91 days (Buesen et al, 2010 and re-examination by Hard GC, 2010).	In addition, the information on the absence of non-neoplastic lesions in kidneys is used for argumentation.

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		consulting capacity. At the request of BASF SE, Ludwigshafen, Germany, in 2009 (January) I examined the mouse kidneys from both the Huntingdon Research Center's 2-year study (HRC, 1983) and the Rallis 18-month study (Rallis, 2003), conducted in CD-1 and Swiss albino mice, respectively. I have reported on my findings in a report to the Company (Hard, 2009). In the HRC study (HRC, 1983), there was a low incidence of renal tubule adenomas in groups exposed to metazachlor - one at 200 ppm (low-dose), 4 at 700 ppm (mid-dose), and 4 at 2500 ppm (high-dose). In the Rallis study (Rallis, 2003), there was one adenoma at the mid-dose of 1000 ppm, and one focus of tubule hyperplasia at the high-dose of 4000 ppm. In 2008 (July 28-30) a Pathology Working Group (PWG) organized by Experimental Pathology Laboratories Inc. of Research Triangle Park, NC, USA, reviewed the proliferative lesions in both studies to judge their relationship to metazachlor exposure. The PWG concluded that the kidney tumors were not treatment-related because of the very low incidence, the lack of a dose response, and the absence of any increase in renal tumors at a higher dose level in the repeat study with mice (Wall, 2008). In my re-evaluation in 2009 (Hard, 2009) I critically examined the tissue slides from		

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		high-dose groups and controls to identify any morphological indicators of renal cell damage. Some evidence of renal tubule injury would be a necessary finding for proposing a mode of action based on sustained toxicity, and resultant compensatory regeneration, caused by the test agent. There was no evidence of cytotoxicity (including tubule basophilia and single cell death), and no increase in mitotic activity in proximal tubule (or other) cells in treated groups of either study. There was also an absence of morphological cell damage in mice sacrificed at 53 weeks in the HRC study, and at earlier time-points. In February, 2010, I examined a sub-chronic cell proliferation study of metazachlor in male mouse kidney, conducted by BASF SE (Hard, 2010). CD-1 mice had been dosed with 0, 200, 700, 2500, and 4000 ppm (doses selected to match those of the 2 chronic studies) for 7, 28 and 91 days and the kidney sections stained immunohistochemically with bromodeoxyuridine (BrdU) as a marker of cell proliferation. This mouse kidney review, in which proximal tubule cell labeling was quantitated, was carried out on coded slides, i.e. without my knowledge of group or animal identity. My evaluation provided no evidence for an increase in cell proliferation, any discursions in treated groups from the control range of labeled cells being trivial,		

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		without a dose response pattern, and of no biological significance. In addition, I examined the companion set of kidney sections that had been stained with hematoxylin and eosin (H&E) and found that at each time point (7, 28, and 91 days) the kidney tissue was normal with no evidence of cytotoxicity. Importantly, there was no variability in nuclear size that would have been indicative of treatment-related cell cycling, in keeping with the negative BrdU result. In the absence of any morphological indicators of cell injury in each one of these studies covering multiple time-points, or increase in cell proliferation in the recent subchronic BrdU study, it can be concluded that the few renal tubule tumors encountered in the HRC study (HRC, 1983) were unrelated to exposure to metazachlor, but of spontaneous origin. As such, this finding would have no relevance for extrapolation to humans. References Hard GC (2009). Expert Re-examination of Renal Histopathology in Carcinogenicity Studies of Metazachlor in Mice, with Particular Reference to Carcinogenic Potential of Metazachlor. Report to BASF SE, Ludwigshafen, Germany, from Gordon C Hard, Tairua, New Zealand. Final Report: February 25, 2009.		

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		Hard GC (2010). Expert Report on Quantitative Assessment of Proximal Tubule Cell Proliferative Activity in Kidneys of Mice administered Metazachlor in the Diet for 7, 28, and 91 Days. Report to BASF SE, Ludwigshafen, Germany, from Gordon C Hard, Tairua, New Zealand. Draft Report submitted, dated* March 14, 2010. HRC (1983). Study BSF 327 - Assessment of Potential Tumorigenic Effects in Prolonged Dietary Administration to Mice (24-Month carcinogenicity Study in CD1 Mice). Huntingdon Research Centre, Huntingdon, Cambridgeshire, England. Final Report: April 27 (BASF Doc ID 1983/091). Rallis (2003). Study No. 1329 – 18-Month Carcinogenicity Study with Metazachlor Technical in Swiss Albino Mice. Rallis Research center, Peenya, Bangalore, India. Final Report: April 24 (TOXI: 1329). Wall HG (2008). Pathology Working Group (PWG) Review of the Carcinogenic Potential of Metazachlor: Kidney Tumors in Male Mice. Pathology Working Group Report to BASF SE, Ludwigshafen, Germany, from Experimental Pathology Laboratories (EPL) Inc, Research Triangle Park, NC, USA. September 16, 2008.		

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		From: Gordon C Hard BVSc, PhD, DSc DACVP. FRCPath, FRCVS, FAToxSci.		
26/04/2010	Germany / Christiane Wiemann / BASF SE and Feinchemie Schwebda GmbH	To allow a thorough evaluation of the data set with regard to tumour incidences potentially relevant for carcinogenic potential the data provided in the Appendix 1 are implicitly to be evaluated. Metazachlor underwent an extensive peer-review by BASF pathologists to address the inconsistent data between the different studies conducted by the two manufacturers. This peer-review was followed by a Pathology Working Group (PWG) evaluation to obtain a final scientific expert conclusion for discrepancies between first assessor and peer-reviewer (here: study pathologists and BASF pathologists). The PWG hereby represent the final conclusion on tumour incidences evaluated according to state of the art diagnostic criteria. CHAPTER 5 - Human Health Hazard Assessment p.37 5.8 Carcinogenicity ….Industry has argued that since the PWG findings were reached by consensus that their review should be considered as definitive. However, although persuasive, since only selected slides were re-examined the UK is of the opinion that it is not appropriate to consider the results as conclusive because some lesions may	All available information was taken into consideration during the development of our proposal, including the opinions of the PWG. As stated above, having already had discussions with the companies concerned where these issues were addressed, we do not intend to amend our CLH report at this stage. We feel that these comments have been submitted to aid RAC discussions and are happy to answer any questions the rapporteur may have	No further comment.

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		have been missed. This concern is highlighted for example, by the fact that the PWG indentified more parafollicular adenomas in the low and mid dose groups than the BASF pathologists in the thyroid of male Wistar rats (although the same criteria were used). Therefore, it is possible, had they examined all the slides, that more adenomas may have been identified in all dose groups. Manufacturers' comment: The argument that relevant findings may have been missed by not investigating all animals is not considered to reflect a realistic concern, due to the following reasons: 1. The Peer Review process of histomorphological slides makes a clear distinction between the role of a peer reviewing pathologist and the role of a PWG. A) A peer reviewing pathologist, who is assessing the relevance of critical findings, will re-evaluate all slides available of the organ or tissue of concern to obtain a complete picture. B) The PWG will clarify all discrepancies between original and peer reviewing pathologist. 2. All critical findings mentioned in the DAR were taken into account by the peer reviewing BASF pathologists. Consequently all available organ slides of organs with critical incidences (liver, thyroid and testes in rat, kidney, liver and	Please see our response to the comments made by Henry Wall	

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		urinary bladder in mice) were re-evaluated by them. The only deviation from this approach was for the lymphoreticular tissue, where only those organs with reported findings of the study pathologists were re-evaluated by the BASF pathologists. With regard to this tumour type the overall incidence of tumours did not result in a concern being equally distributed between control and treated animals. The particular interest was on a specific sub-category introduced by the original study pathologist who diagnosed a "lymphoblastic leukemia". 3. Furthermore additional slides were prepared and evaluated by peer reviewing BASF pathologists for the low and intermediate dose urinary bladder as these dose groups were not evaluated by the study pathologist, given the fact that the low incidences of bladder tumours in the Swiss mice study were considered to be incidental by the study pathologist. Moreover, the additional evaluation of the low and intermediate dose group was considered necessary to further assess the significant diffuse hyperplasia in the high dose group that was missed by the study pathologist. 4. To clarify discrepancies between original diagnosis of the study pathologists and diagnosis of the re-assessment of the peer reviewing BASF pathologists which included additional findings not diagnosed by the study pathologists a PWG was initiated by the		

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		manufacturers, organized and conducted by external consultants / contract research organizations. 5. Aim of a PWG is not to conduct a complete re-evaluation of a study, but to provide a scientific expert opinion for discrepancies between first and re-assessor (here study pathologists and peer-reviewing BASF pathologists). The US-EPA provides the following advise in a pesticide regulation (PR) notice 94-5: "The PWG will review as a minimum, all slides about which there were significantly differing diagnoses between the study and peer review pathologist. 6. The PWG for metazachlor investigated at minimum all slides in the organs of concern that were diagnosed by either the study pathologist or the peer-reviewing pathologist as potentially tumour bearing. Thus, all potential diagnoses of tumours were re-evaluated by them. 7. It is at the discretion of the PWG chairman to further extend a slide re-evaluation by the PWG, if he considers this necessary to obtain a final conclusion. 8. The complete re-evaluation by the PWG is done with coded slides ("blind reading") preventing any bias due to knowledge of dose-groups. 9. With regard to the mentioned thyroid findings please refer to the below given specific explanations (p. 41 Discussion Thyroid) In summary, the peer review process as described above followed an		

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		internationally accepted procedure in the area of toxicologic pathology and ensure an increase in quality and reliability of the histopathological datasets. For further explanation please refer to the attached manufacturers' position on the histopathological peer review process BASF_FCS_1, BASF DocID 2010/1052261 and the manufacturers' position on the histopathological peer review sequence BASF_FCS_2, BASF DocID 2010/1052260. p. 40 5.8.1.1 Rat studies Discussion Liver In Wistar rats, significant increases in adenomas and carcinomas were observed in females at the mid and high dose….. Manufacturers' comment The tumour incidence data do not justify the evaluation of a "significant increase of adenomas and carcinomas" in the mid dose of 2000 ppm. Reasoning: 1. The incidence is low and not statistically significant at all 2. Oppose to the adenoma incidences there is no dose dependent increase from the mid to the high dose 3. Taking into account the combined incidence of adenoma and carcinoma there is a treatment related increase at the high dose only (based on the increased	We did not indicate in our proposal that the increase was 'statistically' significant. However, we felt that the increased incidence of carcinoma in the mid dose	

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		incidence in adenoma). 4. Spontaneous hepatocellular carcinomas are known to occur as age-related lesions in rats and are not a rare tumour type, as it is also reflected by the historical control data of the RITA database for both Wistar and Sprague-Dawley rats (see attached historical control data BASF_FCS_3, BASF DocID 2008/1095200, BASF_FCS_4, BASF DocID 2008/1095199, BASF_FCS_5, BASF DocID 2009/1110093). 5. The PWG did only consider the incidence of hepatocellular adenoma and the combined incidence rate in the high dose females (8000 ppm) as a small treatment related effect. 6. In the study conducted in the other rat strain there is no incidence for liver cell carcinoma determined neither at the mid dose level of 2000 ppm nor at the highest dose level of 6000 ppm that is 3-fold higher than the mid dose level of the study conducted in Wistar rats. In conclusion: In Wistar rats, a small increase in adenomas and combined incidence of adenomas and carcinomas was observed in females at the high dose. Please refer to the manufacturers' position on rat liver carcinogenicity and mode of action (BASF_FCS_6, BASF DocID 2010/1054117). p. 41 5.8.1.1 Rat studies Discussion	(2) was significant as it was higher than both the concurrent (0) and historical controls (0) for that laboratory. A summary of these historical control data is included in Annex 2	

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		Liver ….It is also noted that there was no direct evidence of CAR activation … Manufacturers' comment The Cytochrome P450 iso-enzyme family (CYP) induction of PROD and BROD without induction/with relatively lower induction of EROD reflects a pattern which is in line to phenobarbitone a known inducer of the CYP2B family in rats, mediated by CAR activation (see attached literature BASF_FCS_7, Whysner J, Ross PM, Williams GM (1996) Phenobarbital mechanistic data and risk assessment: enzyme induction, enhanced cell proliferation, and tumour promotion. Pharmacol.Ther. 71 (1-2) 153-191). It could be demonstrated that the enzyme induction is considerably more pronounced in females than in males being in line with sexual hormone counter-regulation in males (see attached study report amendment BASF_FCS_8, BASF DocID 2010/1053010 and attached literature BASF_FCS_9, Hernandez JP et al. (2009)). The conducted comparative study on mRNA expression in female rats treated with either metazachlor (8000 ppm) or phenobarbitone (500 ppm) for 3 or 7 days further supports the suggested mode of action. This study reveals that metazachlor regulates the CYP isoforms 2B1, 2B2, 3C11 and 3A1 - which are known to be under the regulation of CAR (see attached literature BASF_FCS_10,	Please refer to our assessment of the MOA. This can be found in Annex 2. As stated above, having already had discussions with the companies concerned where these issues were addressed, we feel that these comments have been submitted to aid RAC discussions. We are happy to answer any questions the rapporteur may have.	

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		Swales K, Negishi M (2004) CAR, Driving into the future. Minireview Molecular Endocrinology 18 (7) 1589-1598 and BASF_FCS_11, Kodama S and Negishi M. (2006) Phenobarbital confers its divers effects by activating the orphan nuclear receptor CAR. Drug metabolism Reviews 38 (1) 75-87) -similar to phenobarbitone. This clearly indicates that CYP isoforms under the regulation of CAR are similarly up-regulated by phenobarbitone and metazachlor. Moreover the manufacturers conducted further studies to support the phenobarbitone-like CAR mediated MOA by investigating the 1. CAR activation (see attached study reports BASF_FCS_12, BASF DocID 2010/1056091 and BASF_FCS_13, BASF DocID 2010/1056090) 2. S-phase response in female rat liver (see attached study report BASF DocID 2010/1056070) In rat liver treated with metazachlor an accumulation of CAR in the nucleus could be demonstrated by Immuno-Western Blot analysis of the nuclear protein fraction (BASF_FCS_12. BASF DocID 2010/1056091). Moreover in an in vitro transfection reporter gene system in primary rat hepatocytes containing the endogenous rat CAR, CAR mediated induction of Cytochrome 2B1 could be demonstrated on mRNA and activity level after treatment with metazachlor. While in cells transfected with the wild-type	The data on males from study BASF_FCS_8 have been included in the Annex VI CLH report (page 46 Buesen 2010 amendment no 1).	

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		promoter (phenobarbital responsive enhancer module = PBREM) a weak CAR activation could be demonstrated, there was an inhibition noted in cells transfected with a construct that lacks PBREM. The inhibition was attributed to the noted cytotoxicity at that dose level which could have impaired a more pronounced induction in this in vitro system (see attached study report BASF_FCS_13, BASF DocID 2010/1056090). A significant up to 15-fold cell proliferation was determined in female Wistar rats after administration of 8000 ppm metazachlor (BASF_FCS_14, BASF DocID 2010/1056070). In addition liver weight increases and centrilobular hypertrophy of hepatocytes was determined from 7 days onwards. For further explanation and details please refer to the attached manufacturers' position on rat liver carcinogenicity and mode of action (BASF_FCS_6, BASF DocID 2010/1054117). p. 41 5.8.1.1 Rat studies Discussion Liver ….It is also noted that there was no direct evidence of CAR activation and that liver tumour formation was not observed in mice even though they are by far the most sensitive species to phenobarbitone induced carcinogenic response.		

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPSAL ON METAZACHLOR

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		Manufacturers comment: While this species difference is well established for phenobarbitone it must not notably be the same for metazachlor. In a recent review it is demonstrated that other compounds such as pyrethrins and methofluthrin which share the same MOA for liver tumour formation as phenobarbital, liver tumours have been observed in rats and not in mice. This is attributed to differences in metabolism and disposition (see attached literature BASF_FCS_15, Lake BG (2009). Species differences in the hepatic effects of inducers of CYP2B and CYP4A subfamily forms: relationship to rodent liver tumour formation. Xenobiotica 39, 582-596). Metazachlor is extensively metabolized and species differences may occur in metabolization, which could as well explain the species difference in the tumour formation without abnegating the underlying mechanism. CAR mediated effects are described to be more pronounced in females based on the counteractive regulation of male steroid hormones (see attached literature BASF_FCS_9, Hernandez JP, Mota LC, Huang W, Moore DD, Baldwin WS (2009) Sexually dimorphic regulation and induction of P450s by the constitutive androstane receptor (CAR). Toxicology 256 53-64). With metazachlor the slight increased tumour incidences is observed in female animals only and this is supported by the suggested mode of	Please refer to our assessment of the MOA. This can be found in Annex 2.	

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		action. Please refer to the manufacturers' position on rat liver carcinogenicity and mode of action (BASF_FCS_6, BASF DocID 2010/1054117) for further explanation. p.41 5.8.1.1 Rat studies Discussion Liver Conclusion ….Overall there is a clear carcinogenic effect in the liver of female Wistar rats (adenoma and carcinoma) of potential relevance to humans. Manufacturers' comment: The carcinogenic effect observed is considered not to be a "clear" carcinogenic effect. Reasoning: 1. The observed incidences are only slightly above the historical control range and are noted only in one of the studies and only in one sex (females). Thus while a carcinogenic effect is observed in the female Wistar rat at the highest dose tested it is considered to be slight only and therefore not clear. 2. A treatment relation is only given for the high dose incidences in adenoma and there from derived combined incidence of adenoma and carcinoma. The non-statistical significant and non-dose related carcinoma incidences should not be considered treatment related.		Toxicokinetic information was given for the rat. Species differences could only be assumed since no data are available for the mouse. Investigations on CAR-related sexual dimorphism are gained from nonylphenol. Argumentation for Cat 2 considers tumour response in only one sex.

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		3. The PWG did only consider the incidence of hepatocellular adenoma and the combined incidence in the high dose (8000 ppm) female rat as a small treatment related effect. In conclusion: In Wistar rats, a small increase in adenomas and combined incidence of adenomas and carcinomas was observed in females at the high dose. Please refer to the above given comment on the discussion of the rat liver tumours and to the manufacturers' position on rat liver carcinogenicity and mode of action (BASF_FCS_6, BASF DocID 2010/1054117) for further explanation. p.41 5.8.1.1 Rat studies Discussion Thyroid Parafollicular (C-cell) tumours) …. However, as the PWG did not re-examine all the slides, their review is not considered as conclusive and there remains an uncertainty about the significance of the original findings. Manufacturers' comment: The submitters concern does not reflect a realistic concern, for the following reasons: 1. The peer-reviewing BASF pathologists re-evaluated all thyroid slides of that study. Thus, the study and reviewing pathologist have examined all slides of this organ.	We conclude that a carcinogenic response was observed in female Wistar rats. At this stage, due to an absence of established criteria for regulatory acceptance of a phenobarbitone-like mode of action and concerns relating the toxicity of metazachlor to liver cells etc (see Annex 2 for a more detailed summary), we believe it is not currently possible to conclude that the tumours are not relevant to humans.	

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		2. The PWG re-evaluated all slides where a "hyperplasia", an "adenoma" or a "carcinoma" were diagnosed by either the original study pathologist or by the peer-reviewing BASF pathologist or both. The matter of this approach was the matter of appropriate grading the developing sequence from hyperplasia to adenoma and carcinoma. Thus, not only the tumours but also all pre-lesions were re-evaluated by the PWG. 3. This procedure makes it very unlikely that any relevant findings could have been missed and that therefore the PWG "review is not considered as conclusive" and that "there remains an uncertainty about the significance of the original findings" is not realistic. the attached manufacturers' position on the histopathological peer review process BASF_FCS_1, BASF DocID 2010/1052261 and the manufacturers' position on the histopathological peer review sequence BASF_FCS_2, BASF DocID 2010/1052260 for further explanation. p. 42 5.8.1.1 Rat studies Summary of rat data ….In conclusion, in the three available carcinogenicity studies in the rat, metazachlor was shown to have a clear carcinogenic effect in the liver of female Wistar rats (adenoma and carcinoma). All other tumours observed are unlikely to be	These comments do not affect our conclusion as we felt it unlikely that these tumours were treatment related.	

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		treatment related. Manufacturers' comment: As already stated above, the observed incidences are only slightly above the historical control range and are noted only in one of the studies and only in one sex (females). Thus while a tumourigenic effect is observed in the female Wistar rat at the highest dose tested it is considered to be slight only and therefore not clear. Moreover following the PWG conclusion, the treatment relation is only given for the high dose incidences in adenoma and there from derived combined incidence of adenoma and carcinoma. The non-statistical significant and non-dose related carcinoma incidences should not be considered treatment related. Please refer to the above given comment on the discussion of the rat liver tumours. In conclusion, in the three available carcinogenicity studies in the rat, metazachlor was shown to have a small tumourigenic effect at the high dose in the liver of female Wistar rats (adenoma and combined incidence of adenoma and carcinoma). Please refer to the manufacturers' position on rat liver carcinogenicity and mode of action (BASF_FCS_6, BASF DocID 2010/1054117) for further explanation. p. 44 5.8.1.2 Mouse studies Discussion	As stated above, we conclude that a carcinogenic effect was observed in female rats.	

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		Bladder As the original study was conducted in 2003, it is most likely that similar diagnostic criteria to those used by the PWG were employed. It is, therefore, difficult to explain the discrepancy and dismiss the original findings. However, it is noted that the original study pathologist failed to detect the very high incidence of diffuse hyperplasia recorded by all other reviewers, casting some doubt on the original pathologist's findings. As such, for this tumour type, greater weight has been placed on the PWG's findings. However, as not all slides were examined by the PWG it is considered imprudent to dismiss the original study pathologist's findings completely Manufacturers' comment: The submitters concern that not all slides were assessed by the PWG and that it is therefore "imprudent to dismiss the original study pathologist's finding" does not reflect a realistic concern, for the following reasons: 1. Study pathologists and peer reviewing LPT and BASF pathologists have reviewed all available slides from the urinary bladder. In addition, BASF pathologists have examined the urinary bladder of all intermediate groups. Thus, the extended peer-review of the BASF pathologist is more complete than the examination of the original study pathologist.		

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		2. Furthermore, both, LPT and BASF reviewing pathologists detected one additional papilloma in one male control animal as well as a significant incidence of diffuse hyperplasia in high dose group males and females not diagnosed by the original study pathologist. These findings were also confirmed by the PWG. 3. The PWG re-evaluated all slides where papilloma or carcinoma were assessed by either the original study pathologist or by the peer-reviewing LPT or BASF pathologist or all. The PWG also re-evaluated most of the diffuse hyperplasia findings in the high dose group animals, a finding that is considered to be treatment related but not to be a precursor of tumour formation. This finding with a significant incidence was not diagnosed by the original study pathologist. Thus, arguing the PWG assessment was incomplete and that it is therefore "imprudent to dismiss the original study pathologist's findings completely" does not adequately reflect the results of the peer-review process and the complete data set. Please refer to the manufacturer' position on histopathological peer review sequence BASF_FCS_2, BASF DocID 2010/1052260 for further explanation. p. 44 5.8.1.2 Mouse studies Discussion Bladder	These comments do not affect our proposal as we concluded that the bladder tumours were not treatment related.	

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		"In mechanistic studies, no evidence of microcrystallisation was detected in the bladder of mice (see section 5.8.5) ruling out this species specific mode of action. Metazachlor was found to increase cell proliferation in the bladder of both MF1 and CD1 mice, which is consistent with the findings observed in the study." Manufacturers' comment: It should be specified that the finding "is consistent with the finding diffuse hyperplasia of the transitional cell epithelia observed in the study ". Reasoning: 1. The increased cell proliferation is closely linked to the histomorphological finding of a "diffuse hyperplasia" in both studies. 2. A diffuse hyperplasia is not considered to represent a precancerous lesion but rather represents an adaptive, reactive (protective) mechanism on various irritating environments that normally not progress to tumour. 3. A focal hyperplasia instead may be considered a precancerous lesion. However, neither CD-1 nor Swiss mice showed any suspect incidence of a focal hyperplasia in the urinary bladder of treated animals. 4. This is further supported by the aspect that the increase in cell proliferation and diffuse hyperplasia of the transitional cell epithelia is more pronounced in the non tumour bearing CD-1 mouse strain.		

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		Please refer to the manufacturer' explanation in BASF_FCS_16, BASF DocID 2009/1109594. p.45 5.8.1.2 Mouse studies Discussion Kidney …. However, historical control data for the laboratory presented in the PWG report showed that the adenoma incidence was above the historical control range, while the carcinoma was well within the range…. Manufacturers' comment: The historical control incidences discussed in here should be presented in the table above and they should be included as reference (see attached historical control data BASF_FCS_17, BASF DocID 2008/1095170). p. 45 5.8.1.2 Mouse studies Discussion Kidney …The PWG more or less confirmed the original study findings. The only difference was that they identified an additional adenoma in the mid and high dose groups and did not confirm the presence of the carcinoma… Manufacturers' comment: This wording that "they (PWG) identified	We agree that the hyperplasia was mainly diffuse and are happy to indicate so if required. However, these comments do not affect our proposal as we concluded that the bladder tumours were not treatment related.	

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		an additional adenoma" is misleading. It should instead read that a "carcinoma" was downgraded by the PWG to an "adenoma". Moreover, the PWG concluded that: "The kidney tumours observed in male mice in the HRC study BSF 327/82389 are not considered to be treatment-related due to the very low incidence, lack of a dose-response relationship, no increased incidences at higher dose levels in a second long-term mouse study (Rallis Study No.: 1329), and the higher spontaneous tumour rate which is known to occur in male mice." Please refer to the manufacturers' position on the treatment relationship of the mouse kidney tumours (BASF_FCS_18, BASF DocID 2010/1054118) for further explanation. p. 45 5.8.1.2 Mouse studies Discussion Kidney …. Nonetheless, since the increase of adenoma was confirmed by the PWG, was dose-related and the incidence at the top and mid dose was above the historical control range... Manufacturers' comment The argument given above that the increase in adenoma was dose-related is not correct. The incidences in the male kidney did not show a dose-response	Reference to the historical control incidence is included in Annex 2 where the range is provided.	

<table>
<tr><th>Date</th><th>Country/ Person/Organisation/ MSCA</th><th>Comment</th><th>Response</th><th>Rapporteur’s comment</th></tr>
<tr><td></td><td></td><td>relationship. A more than 3-fold increase in dose from 700 to 2500 ppm did not result in an increase in tumour incidence at all.

Please refer to the manufacturers' position on the treatment relationship of the mouse kidney tumours (BASF_FCS_18, BASF DocID 2010/1054118) for further explanation.

p.46
5.8.1.2 Mouse studies
Discussion
Kidney
…. Nonetheless, since the increase of adenoma was confirmed by the PWG, was dose-related and the incidence at the top and mid dose was above the historical control range, these results suggest a weak carcinogenic response in the kidneys of CD-1 mice (an increase in benign adenomas in one sex and one strain) of potential relevance to humans…

Manufacturers' comment:
Deviating from the dossier submitter’s position the manufacturers consider the kidney tumour incidences not to be treatment related following the conclusion of the PWG as presented above and re-examination by an internationally recognized expert pathologist on renal toxicity (see section 5.8.5 Other relevant information).
Please refer to further comments to</td><td>The incidence of adenoma is reproduced here:
<table><tr><td>Original study</td><td>0</td><td>1 (2%)</td><td>3 (6%)</td><td>4 (8%)*</td></tr></table></td><td></td></tr>
</table>

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		section 5.8.5 Other relevant information below and to the manufacturers' position on the treatment relationship of the mouse kidney tumours (BASF_FCS_18, BASF DocID 2010/1054118) for further explanation. p. 46 Summary of mouse data …. In conclusion, in the two available mouse carcinogenicity studies (one in Swiss mice and one in CD-1 mice), metazachlor appeared to have a weak carcinogenic effect in the kidney only. In this organ, only benign tumours were observed and the effect was inconsistent between both strains and sexes. … Manufacturers comment: Deviating from the dossier submitter's position the manufacturers consider the kidney tumour incidences not to be treatment related following the conclusion of the PWG as presented above and re-examination by an internationally recognized expert pathologist on renal toxicity (see section 5.8.5 Other relevant information). To substantiate this position further investigations in mice were initiated. Please refer to further comments to section 5.8.5 Other relevant information below and to the manufacturers' position on the treatment relationship of the mouse kidney tumours (BASF_FCS_18, BASF DocID 2010/1054118) for further	Internal review: 0 / 1 (2%) / 3 (6%) / 4 (8%) PWG review: 0 / 1 (2%) / 4 (8%) / 4 (8%) Since we have had previous discussion with the companies concerned, we feel that these comments have been submitted to aid RAC discussions. Against this background, we do not plan to change our position, but are happy to help the	

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		explanation. p.46 5.8.5 Other relevant information Table 5.17 Additional information relevant for carcinogenicity Microcrystallisation in the urinary bladder and enzyme induction in the liver and kidney of rat. Manufacturers' comment: In addition to the Benzoxyresorufin-O-debenzylase (BROD) also the Pentoxyresorufin-O-dependentylase (PROD) activity was increased in female rats. While the levels in the control group animals were below the detection limits. The levels for liver from animals treated with 8000 ppm metazachlor was about 63.327 pmol Resorufin/min/mg protein and thus in a comparable range to the BROD levels of about 80.736 pmol Resorufin/min/mg protein. In treated kidney the levels were less pronounced being 0.145 pmol Resorufin/min/mg protein PROD activity compared to 0.764 pmol Resorufin/min/mg protein BROD activity. The PROD activity increase both in liver and kidney compared to the control group was considered to be treatment related. To further substantiate the relationship between enzyme induction and potential tumour formation in female rats, a comparative assessment on enzyme activity in livers of males has been	rapporteur in the development of their proposal and answer any queries they may have.	

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		conducted (see attached study report amendment, BASF_FCS_19, BASF DocID 2010/1053010). In here the induction of PROD and BROD without consecutive induction of EROD by administration of 8000 ppm metazachlor could be confirmed. The effect in males was however less pronounced than the effect in females (10-fold increase by metazachlor in males compared to more than 100-fold increase in females. This sex difference is in line with the tumour formation and in line with the sex specific regulation of the constitutive androstane receptor (CAR) described in the literature, being the suggested mode of action for metazachlor liver tumour formation in female Wistar rats. CAR mediated effects are described to be more pronounced in females based on the counteractive regulation by male steroid hormones in males (BASF_FCS_9, Hernandez JP, Mota LC, Huang W, Moore DD, Baldwin WS (2009) Sexually dimorphic regulation and induction of P450s by the constitutive androstane receptor (CAR). Toxicology 256 53-64). A detailed discussion of the CAR mediated enzyme induction its role in xenobiotic detoxification and phenobarbitone like liver cell proliferation is provided in the attached manufacturers' position on rat liver carcinogenicity and mode of action (BASF_FCS_6, BASF DocID 2010/1054117). p. 47	Since we have previous discussion with the companies concerned, we feel that these comments have been submitted to aid RAC discussions. Against this background, we do not plan to change our position, but are happy to help the rapporteur in the development of their proposal and answer any queries they may have.	

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		5.8.5 Other relevant information Table 5.17 Additional information relevant for carcinogenicity mRNA Analysis of Liver tissue form Rat treated for 3 and 7 days with Phenobarbitone or BAS479H (metazachlor) Conclusion Metazachlor and phenobarbitone increase the mRNA levels of certain cytochrome P450 isoforms similarly, whereas differences were more pronounced for phase II metabolising enzymes Manufacturers comments: The investigated Cytochrome P450 Iso-forms of the CYP2B isofamily are known to be under the regulation of the constitutive androstenone receptor CAR as well established in the literature (BASF_FCS_10, Swales K, Negishi M (2004) CAR, Driving into the future. Minireview Molecular Endocrinology 18 (7) 1589-1598, BASF_FCS_11, Kodama S and Negishi M. (2006) Phenobarbital confers its divers effects by activating the orphan nuclear receptor CAR. Drug metabolism Reviews 38 (1) 75-87). A detailed discussion of the CAR mediated enzyme induction its role in xenobiotic detoxification and phenobarbitone like liver cell proliferation is provided in the attached manufacturers' position on rat liver carcinogenicity and mode of action (BASF_FCS_6, BASF DocID 2010/1054117). Thus, demonstrating the	This should be reference to BASF_FCS_8 and the PROD, BROD and EROD data for males have been included in the Annex VI CLH report.	

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		enzyme induction of these enzymes on mRNA as well as functional level similar to phenobarbitone provides a clear link to CAR activation. Moreover the manufacturers meanwhile conducted studies where the relation of metazachlor treatment to CAR activity has been assessed in rats (see attached study reports BASF_FCS_12, BASF DocID 2010/1056091 and BASF_FCS_13, BASF DocID 2010/1056090 and manufacturers' position on rat liver carcinogenicity and mode of action (BASF_FCS_6, BASF DocID 2010/1054117). In rat liver treated with metazachlor accumulation of CAR in the nucleus could be demonstrated by Immuno-Western Blot analysis of the nuclear protein fraction (BASF_FCS_12, BASF DocID 2010/1056091). Moreover in an in vitro transfection reporter gene system in primary rat hepatocytes containing the endogenous rat CAR, CAR mediated induction of Cytochrome 2B1 could be demonstrated on mRNA and activity level after treatment with metazachlor. While in cells transfected with the wild-type promoter (phenobarbital responsive element = PBREM) a weak CAR activation could be demonstrated, there was an inhibition noted in cells transfected with a construct that lacks PBREM. The inhibition was attributed to the noted cytotoxicity at that dose level which could counteract a more pronounced induction in this in vitro	Since we have already had discussions with the companies concerned, we feel that these comments have been submitted to aid RAC discussions. Against this background, we do not plan to change our position, but are happy to help the rapporteur in the development of their proposal and answer any queries they may have.	

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		system (see attached study report BASF_FCS_13, BASF DocID 2010/1056090) and the manufacturers' position on rat liver carcinogenicity and mode of action (BASF_FCS_6, BASF DocID 2010/1054117). Another conclusion that can be drawn from the study results which is not adequately presented in the Annex VI report is that no early inflammatory effects related to liver toxicity are induced by metazachlor. This was addressed in the study by investigating genes of the extracellular matrix turnover (e.g. Col1A1, 1A2, 3A1 and Fibronectin). These genes were not significantly affected by either metazachlor or phenobarbitone treatment indicating that no pro-inflammatory signalling occurred in the hepatic cells. Please refer also to the attached study report BASF_FCS_19, BASF DocID 2010/1043666 and the manufacturers' position on rat liver carcinogenicity and mode of action (BASF_FCS_6, BASF DocID 2010/1054117). p. 47 5.8.5 Other relevant information Table 5.17 Additional information relevant for carcinogenicity Thyroid hormone study …Conclusion Failure to see any changes in T3 or T4 levels questions the hypothesis that the		

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		mode of action is the same as that of phenobarbitone. Manufacturers' comment: The failure to see any changes in T3 or T4 levels does not necessarily question the hypothesis that the mode of action is the same as phenobarbitone. The thyroid hormone homeostasis is well regulated by the negative compensatory feed-back mechanism on the hypothalamic / pituitary gland axis mediated via TSH aiming to restore the physiological hormone levels. An increase in TSH levels after metazachlor treatment was shown. Moreover, the increase of UDP-glucuronyltransferase activities similar to phenobarbitone were as well demonstrated in the 14-day enzyme induction study as in the mRNA analysis of liver tissue from rat treated for 3 and 7 days with phenobarbitone or metazachlor (see table 5.17). It should be noticed that the metazachlor related effects on the follicular cells of the thyroid are not very pronounced, which might explain that also the effects on hormone homeostasis are less pronounced and clear. p. 47 5.8.5 Other relevant information Table 5.17 Additional information relevant for carcinogenicity Re-examination of renal histopathology in carcinogenicity studies of metazachlor in		

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		mice Manufacturers' comment: The re-examination of the internationally recognized expert pathologist on renal toxicity gave no indication for any underlying toxicological mode of action that could be related to the slight increase in renal tubule tumours in the high dose group males of the CD-1 mice, not seen in the second mouse carcinogenicity study at even higher dose levels. Examination of the high-dose male kidneys from each of the two mouse carcinogenicity studies revealed no evidence of cytotoxicity or mitotic activity in either case, covering a wide span of time for individual animals. No treatment related toxicological effect could be established by him that could link the kidney tumour formation to the treatment of metazachlor and based on a weight of evidence approach he came to the conclusion that the tumours are not treatment-related. In addition the manufacturers' initiated an additional evaluation of the kidneys form the recently conducted 90-day S-phase study in CD-1 mice (BASF_FCS_20, BASF DocID 2010/1055081). The kidneys were qualitatively assessed for renal toxicity on H.E. stained slides and quantitatively assessed for cell proliferation based on blind reading of BrdU stained slides. In conclusion, the determined slight increase of cell	Since we have had discussions with the companies concerned, we feel that these comments have been submitted to aid RAC discussions. Against this background, we do not plan to change our position, but are happy to help the rapporteur in the development of their proposal and answer any queries they may have.	

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		proliferation after 28 and 91 days of treatment – although considered as a treatment-related effect – was of no toxicological relevance, as any treatment-related structural lesions in the kidney parenchyma were missing, biologically relevant kidney weight changes were not present and a clear dose-dependency was not observed, after all three periods of treatment. For further explanation please refer to the study report BASF_FCS_20, BASF DocID 2010/1055081 and the manufacturers' position on kidney tumour formation in mice (BASF_FCS_18, BASF DocID 2010/1054118). In addition this expert pathologist re-examined the additionally conducted S-phase response study in male CD-1 mice kidneys to seek for evidence for a mode of action underlying renal tubule tumour development. Hard concluded that this study has conclusively demonstrated that metazachlor exerts no pathological effects on mouse kidney. Consequently, the few renal tubule tumours encountered in previous chronic studies should be considered to be of spontaneous origin and not related in any way to test article administration. For further explanation please refer to the attached report BASF_FCS_21. BASF DocID 2010/1054128 and the manufacturers' position on kidney tumour formation in mice BASF_FCS_18, BASF DocID	Since we have had discussions with the companies concerned, we feel that these comments have been submitted to aid RAC discussions. Against this background, we do not plan to change our position, but are happy to help the rapporteur in the development of their proposal and answer any queries they may have.	

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		2010/1054118. p. 49 5.8.5 Other relevant information ... A number of mechanistic studies have been conducted. Although for some tumour types in the rat (namely the liver) there were some indications of species specific mechanisms, there was insufficient evidence to support them conclusively. Manufacturers' comment: As indicated above the manufacturers conducted further studies to substantiate the CAR mediated phenobarbitone like mode of action on liver tumour formation by demonstrating direct CAR activation of metazachlor (BASF_FCS_12, BASF DocID 2010/1056091 and BASF_FCS_13, 2010/1056090) and quantifying the induced cell proliferation in metazachlor treated rat liver (BASF_FCS_14, BASF DocID 2010/1056070). Please refer to the above given comments and the manufacturers' position on rat liver carcinogenicity and mode of action (BASF_FCS_6, BASF DocID 2010/1054117). p. 49 5.8.5 Other relevant information ... For the other tumour types no clear modes of action were identified. Manufacturers' comment:		

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		The aspect that manufacturers attempts to establish toxicological effects that could be linked to the kidney tumour formation failed should raise doubts on the treatment relationship of these tumours. p. 49 5.8.6 Summary of discussion of carcinogenicity …In the rat, metazachlor was shown to have a clear carcinogenic effect in the liver (adenomas and carcinomas).... Manufacturers comment: As already stated above, the observed incidences are only slightly above the historical control range and are noted only in one of the studies and only in one sex (females). Thus, while a tumourigenic effect is observed in the female Wistar rat at the highest dose tested it is considered to be slight only and therefore not clear. Moreover following the PWG conclusion, the treatment relation is only given for the high dose incidences in adenoma and there from derived combined incidence of adenoma and carcinoma. The non-statistical significant and non-dose related carcinoma incidences should not be considered treatment related. Please refer to the above given comment on the discussion of the rat liver tumours and the manufacturers' position on rat liver carcinogenicity and mode of action (BASF_FCS_6, BASF DocID 2010/1054117).		

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		p. 49 5.8.6 Summary of discussion of carcinogenicity …However, on consideration of all the available data, there are a number of factors that indicate classification in category 3 would be more appropriate. Most significantly, there is the lack of genotoxicity seen with metazachlor in in vitro and in vivo studies. Also, the carcinogenic response in the mouse is very weak with small increases limited to one site (kidney), one sex and one strain and of benign nature… Manufacturers' comment It is the manufacturers' opinion that the available data set does not necessarily warrant classification with regard to carcinogenicity. With regard to carcinogenic potential of metazachlor the slight incidences of benign kidney adenomas of male mice in one of the two submitted studies are not considered treatment related as they are not dose-dependent, not seen in a second study at even higher dose levels and not related to any indication of kidney structural alterations. The slight increased incidence in benign liver adenomas of female Wistar rats at the highest dose is considered most likely treatment related but caused by a non-genotoxic indirect mechanism based on a phenobarbitone-like enzyme induction and cell	These further studies are summarised in Annex 2 to this table BASF_FCS_12 (Wang, 2010), BASF_FCS_13 (Neuschafer-Rube and Puschel, 2010) and BASF_FCS_14 (Buesen et al, 2010).	

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		proliferation mediated by CAR activation which is not considered relevant for humans. It is the manufacturers' opinion that when applying the criteria and considerations of the CLP Regulation 1272/2008 a classification of metazachlor Carc. 2 H351: Suspected of causing cancer is not warranted for the following reasons. The studies conducted do not demonstrate limited evidence (suspected human carcinogen) when applying the given criteria: The slight increased incidence was observed in one species rat only The slight increased incidences was observed in one of the two studies only The slight increase incidence was observed in one sex females only The slight increased incidences in rat liver tumours was seen at high dose only with evidence of excessive toxicity (10% retardation in weight gain) There is no evidence for malignant neoplasm or progression to malignancy; only slightly increased benign tumour incidences are under consideration There is no multi-site response in the rat There is no mode of action identified with relevance for humans p. 67-p. 68 References		

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Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		Manufacturers' comments: The references for the manufacturers histopathological peer-review, the PWG reports and the historical control data are missing and should be added 1. Anonymous (2008a) To whom it may concern: BASF, Makhteshim-Agan and Feinchemie position on proposed R40 classification of Metazachlor - detailed assessment, dated March 25, 2008, BASF_FCS_22, BASF DocID 2008/1095109 2. Wiemann C and Kaufmann W (2009) Metazachlor - Explanation on open points raised by RMS United Kingdom in the draft Annex VI Report: Proposal for harmonised classification and labelling including corrected tables and revised historical control data, BASF_FCS_16, BASF DocID 2009/1109594 3. Wall HG (2008a) Pathology Working Group (PWG) Review of the Carcinogenic Potential of Metazachlor: Liver and Thyroid Gland of Sprague-Dawley and Wistar Rats. HRC Study No BSF 326/8226/2 reissued 11 May 1983, HRC Study No. BSF 340/82449/2 reissued 9 May 1983, Rallis Study No. TOXI-1328 C:C_R; 27 May 2002 - Pathology Working Group Report. Experimental Pathology Laboratories (EPL) Study 717-009, Final report: September 16, 2008, BASF_FCS_23, BASF DocID 2008/1070697.		

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		4. Wall HG (2008b) Pathology Working Group (PWG) Review of the Carcinogenic Potential of Metazachlor: Interstitial Cell (Leydig) Cell Tumours of Sprague-Dawley Rats. HRC Study No BSF 326/8226/2 reissued 11 May 1983 - Pathology Working Group Report. Experimental Pathology Laboratories (EPL) Study 717-009, Final report: September 16, 2008, BASF_FCS_24, BASF DocID 2008/1070691 5. Wall HG (2008c) Pathology Working Group (PWG) Review of the Carcinogenic Potential of Metazachlor: Proliferative Lesions in the Urinary Bladder in Swiss Albino Mice. Rallis Study No. 1329 (24 April, 2003) - Pathology Working Group Report. Experimental Pathology Laboratories (EPL) Study 717-009, Final report: September 16, 2008, BASF_FCS_25, BASF DocID 2008/1070699 6. Wall HG (2008d) Pathology Working Group (PWG) Review of the Carcinogenic Potential of Metazachlor: Lymphoreticular Tumours in Male CD-1 (Charles River) Mice. HRC Study No BSF 327/82389 (27 April, 1983) - Pathology Working Group Report. Experimental Pathology Laboratories (EPL) Study 717-009, Final report: September 16, 2008, BASF_FCS_26, BASF DocID 2008/1070700 7. Wall HG (2008e) Pathology Working Group (PWG) Review of the Carcinogenic Potential of Metazachlor: Kidney	As already indicated to aid the rapporteur we have listed the data submitted by industry (refer to Annex 3) and included a comment to indicate what action has been	

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Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		Tumours in Male Mice. HRC Study No BSF 327/82389 (27 April, 1983) and Rallis Study No. 1329 (24 April, 2003) - Pathology Working Group Report. Experimental Pathology Laboratories (EPL) Study 717-009, Final report: September 16, 2008, BASF_FCS_27, BASF DocID 2008/1070692 8. Wall HG (2008f) Pathology Working Group (PWG) Review of the Carcinogenic Potential of Metazachlor: Liver Tumours of CD-1 (Charles River) Female Mice. HRC Study No BSF 327/82389 issued 27 April 1983 - Pathology Working Group Report. Experimental Pathology Laboratories (EPL) Study 717-009, Final report: September 16, 2008, BASF_FCS_28, BASF DocID 2008/1070698 9. Anonymous (2008b) Historical Histopathology Data Long term studies CD rats, Liver Tumours, Thyroid Tumours. Huntingdon Life Science issued February 11, 2008, BASF_FCS_29, BASF DocID 2008/1095179 10. Anonymous (2008c) Historical Histopathology Data Long term studies CD rats, Testes - Interstitial Cell Tumours. Huntingdon Life Science issued March 7, 2008, BASF_FCS_30, BASF DocID 2008/1095180 11. Anonymous (2008d) Historical Histopathology Data Long term studies CD-1 Mice, Lymphoreticular Tumours, Kidney Tumours, Urinary Bladder Tumours. Huntingdon Life Science issued	taken with this information. It should be noted that some of the information referenced here was already included in the original submission. All of the submitted data have been attached to the IUCLID. In addition, please see the attached Annex 2 which contains a summary of some of these new data submitted in support of a phenobarbitone-like mode of action. The studies summarised include: • The effects of Metazachlor on CAR activation: a mechanism for the observed CYP2B induction (Wang, 2010) • Induction of the CYP2B1 promoter by metazachlor-dependant CAR (NR1I3) activation in primary cultures of rat hepatocytes (Neuschafer-Rube and Puschel, 2010) • S-phase response study in Wistar Rats administration in the diet for 3, 7, 14 and 28 days (Buesen et al, 2010) • S-phase response study in CD-1 mice administration in the diet for 7, 28 and 91 days (Buesen et al, 2010) and re-examination of data (Hard GC, 2010)	

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		February 26, 2008, BASF_FCS_17, BASF DocID 2008/1095170 12. Anonymous (2008e) Historical Histopathology Data Long term studies CD-1 Mice, Liver - Hepatocellular Tumours. Huntingdon Life Science issued March 10, 2008, BASF_FCS_32, BASF DocID 2008/1095169 13. Anonymous (2008f) Historical Data 38 Combined Chronic Toxicity and Carcinogenicity Study in Rats. 38.16: Histopathological (Non-Neoplastic & Neoplastic) Findings of Combined Fates. Liver, Kidney, Urinary Bladder, Thyroids. Advinus Therapeutics HD-C.C.R 38/16/Edition 6/2008 BASF_FCS_33, BASF DocID 2008/1095172 14. Anonymous (2008g) Historical Data 40 Carcinogenicity Study in Swiss Albino Mice. 40.9: Histopathological (Non-neoplastic and Neoplastic) Findings of Combined Fate Mice. Kidneys, Urinary Bladder. Advinus Therapeutics HD-CARCI-M 40.9/Edition 6/2008 BASF BASF_FCS_34, DocID 2008/1095174 15. Anonymous (2008h) Historical Data 40 Carcinogenicity Study in Swiss Albino Mice. 40.9: Histopathological (Neoplastic) Findings of Combined Fate Mice. Liver. Advinus Therapeutics HD-CARCI-M 40.9/Edition 6/2008 BASF_FCS_35, BASF DocID 2008/1095173 16. Anonymous (2008i) Lesion-related Incidence Data - Rat SPRD, Liver: Adenoma, hepatocellular. Report created: 21-Jan-2008, BASF_FCS_3, BASF		

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		DocID 2008/1095200 17. Anonymous (2008j) Lesion-related Incidence Data - Rat SPRD, Liver: Carcinoma, hepatocellular. Report created: 20-Feb-2008, BASF_FCS_4, BASF DocID 2008/1095199 18. Anonymous (2008k) Lesion-related Incidence Data - Rat SPRD, Thyroid gland: Adenoma, C-cell. Report created: 21-Jan-2008, BASF_FCS_38, BASF DocID 2008/1095195 19. Anonymous (2008l) Lesion-related Incidence Data - Rat SPRD, Thyroid gland: Adenocarcinoma, follicular cell, Adenoma, follicular cell, Carcinoma, C-cell. Report created: 20-Feb-2008, BASF_FCS_39, BASF DocID 2008/1095194 20. Anonymous (2008m) Lesion-related Incidence Data - Rat SPRD, Testis: Adenoma, Leydig cell, Carcinoma, Leydig Cell, Hyperplasia, Leydig cell - Focal/multifocal, Hyperplasia, Leydig cell -Diffuse (severe). Report created: 11-Mar-2008, BASF_FCS_40, BASF DocID 2008/1095196 21. Anonymous (2009) Lesion-related Incidence Data - Rat Wistar, Liver Adenoma, hepatocellular, Carcinoma, hepatocellular. Report created: 05-Oct-2009, BASF_FCS_5, BASF DocID 2009/1110093 22. Anonymous (2008n) Lesion-related Incidence Data - Mouse CD-1, Kidney, Adenoma. Report created: 21-Jan-2008, BASF_FCS_38, BASF DocID		

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Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		2008/1095190 23. Anonymous (2008o) Lesion-related Incidence Data - Mouse CD-1, Kidney, Carcinoma. Report created: 20-Feb-2008, BASF_FCS_39, BASF DocID 2008/1095201 24. Anonymous (2008p) Lesion-related Incidence Data - Mouse CD-1, Liver, Adenoma, hepatocellular, Carcinoma, hepatocellular. Report created: 11-Mar-2008, BASF_FCS_40, BASF DocID 2008/1095191 p. 73 Table 2 It is unclear whether the historical control data was derived form 18 month or 2 year studies Manufacturers' comments: The provided historical control data table gives the exact study duration for every single study. Most of the studies lasted for two years (>= 104 weeks). Some studies with shorter duration but > 18-month are included. As the metazachlor study was conducted for two year the inclusion of historical control data from studies with shorter duration will not bias the database.		
26/04/2010	Spain / Elina Valcare / MSCA	p 49 Summary and discussion of carcinogenicity The Spanish CA supports the proposed classification of Metazachlor as category 3 carcinogen, R40 based on Directive 67/548/EEC and as category 2 carcinogen;	Thank you for these comments	

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		H351 based on Regulation EC/1272/2008. Metazachlor is extensively metabolized and species and sex differences may occur in metabolization, which could explain the species difference in the tumour formation. The increase of renal tubule adenomas observed in male CD-1 mice was dose-related and the incidence at the top and mid dose was above the historical control range. Although there was no evidence of sustained toxicity and/or regeneration, suggesting that the hepatocelular kidney tumors observed were unlikely to have arisen through a mechanism involving cytotoxicity or mitotic activity, a mode of action was not identified. Therefore, the results suggest a weak carcinogenic response (an increase in benign tumours inconsistent between strains and sexes) of potential relevance to humans. In female Wistar rats metazachlor was shown to have a clear carcinogenic effect in the liver (adenomas and carcinomas) of potential relevance to humans. Two years treatment with the two higher doses of Metazachlor produced hepatocellular carcinomas above the range of historical control incidences. The incidence of hepatocellular adenomas was increased above the range of historical control incidences at the highest dose. In contrast, Metazachlor was not carcinogenic in the		

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		liver in male or female CD-1 mice. The MOA of Metazachlor-induced liver tumours is postulated by the manufactures to involve the induction of certain cytocrhrome P450 iso-forms as CYP 2B1, 2B2, 2C11 and 3A1, genes known to be under the regulation of the constitutive androstane receptor (CAR), similar to other nongenotoxic substances, liver CYP2B inducer/CAR activator, such as Phenobarbital (PB). PB is a chemical for which there is strong epidemiological data supporting non-carcinogenicity in humans. There is also significant evidence that increased cell proliferation observed in PB-induced liver tumours in rodents, does not occur in the human liver. One finding consistent with a PB-like response are the induction of CYP450 of the 2B family, confirmed by the results of gene expression studies showing higher 2B mRNA levels after administration of Metazachlor. Other findings consistent with a PB-like response are observations from repeat doses studies of increased liver weight and centrilobular hepatocellular hypertrophy. The development of altered hepatic foci is also a key event in the MOA for Phenobarbital-induced liver tumors. Like PB, the appearance of such foci, adenomas and carcinomas occurred only after chronic administration of Metazachlor.		

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		However, data for concordance analysis with PB are limited. There are a number of data gaps, such as the lack of available data regarding CAR involvement in the induction of CYP2B isoforms following Metazachlor exposure and there is no data regarding the concordance of key events between rat and humans. CAR dependency of PB-induced CYP2B induction was confirmed as PB does not produce liver tumours in CAR knockout mice. Although a CAR knockout rat has not to date been developed, the role of CAR in the CYP2B induction for Metazachlor has not been determinated using a recently developed RNA interference (RNAi) technique in CAR knockdown rat hepatocytes. Consequently, CAR dependency of this effect has not been confirmed. The MOA (Mode of Action) for liver tumor formation by Phenobarbital involves an increased of cell proliferation. An S-Phase Response Study (using BrdU Stained cells) to determining whether metazachlor induces cell proliferation in the liver of Wistar rats was not carried out and the CAR dependency of this effect has not been established. There are no data on the effects of Metazachlor on apoptosis in the liver of rats and inhibition of apoptosis is		

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		considered a key event in the MOA for Phenobarbital-induced liver tumours. Besides, the administration of Metazachlor did not result in an enzyme induction profile in the CD-1 mice liver similar to that observed with phenobarbital. To define a MOA in liver, it is critical to ensure that other MOAs do not contribute significantly to hepatocarcinogenesis. There was no evidence of hepatocellular cytotoxicity (necrosis). However, it is important to ensure that DNA reactivity, other possible MOA for the induction of liver tumours in rats, is not the source of the tumour findings. In this sense, there is no data, such as DNA adducts analysis in liver cells, to assess whether hepatocelular tumours seen are attributable to specific mutagenic events. For this compound, there is not robust data for a PB-like MOA and there is not a satisfactory demonstration that other molecular mechanisms are not relevant. Relationships between metazachlor activation pathways and their involvement in carcinogenesis should be further established. Therefore, based on the data available, the mode of action for formation of liver tumours in Wistar rats remains unclear, which leads to the conclusion that the MOA for liver tumours in rat could be applicable to man.		

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPSAL ON METAZACHLOR

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		Although metazachlor produced tumours in rat liver and renal tumours in mice with low incidence and only at high exposure levels. The results from the supplementary studies are not sufficient to eliminate the concern for the relevance these tumours to humans. Given the uncertainties and considering the structural similarity with a known carcinogen like alachlor, the classification regarding carcinogenicity can not be ruled out. On balance, we considered that the proposed classification as Carc. Cat 3; R40 under Directive 67/548/EEC and Carc 2; H351 under the regulation EC/1272/2008 is appropriate.		
21/04/2010	Belgium / Frederic Denauw / MSCA	*Health effects* <u>CLH proposal Human Health (BE)</u> *Proposed classification based on CLP criteria* Signal word : warning Classification : Carc. 2 Skin Sens. 1 H-statements : H351 : Suspected of causing cancer H317 : may cause an allergic skin reaction *Proposed classification based on Directive 67/548/EEC criteria*	Thank you for these comments	Noted.

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		Class of Danger Xn: Harmful R-Phrases R43: May cause sensitisation by skin contact R40 (Carc. Cat 3): Limited evidence of a carcinogenic effect <u>Preliminary remark:</u> In the CLH proposal of RMS UK, tables with neoplastic findings were presented. However, the data pertain on the original assessment performed by the study pathologist. In the meanwhile, the notifier presented data from an independent pathology working group (PWG). The PWG data were not reproduced in the CLH report itself (only in an appendix), and are presented hereunder. RMS highlighted that only selected slides (i.e. slides where neoplastic findings were assessed by either the original pathologist or by the peer-reviewing BASF pathologist) were re-examined by the PWG. Therefore, the findings reached by consensus were considered inappropriate by the RMS, as some lesions could have been missed. As a response, notifier brings under attention that all critical findings (liver, thyroid and testes in the rat, and liver, kidney and urinary bladder in mouse) have been re-evaluated internally by pathologists (the findings were comparable to those observed afterwards by the PGW). Therefore, it is the opinion of BE that the PGW findings could well be considered.	Appendix 1 to the CLH report contains the summary of the PWG findings. Due to the amount of information we felt it would be clearer to present the information in this way.	

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		Except for the C-cell lesions in the SD rats, and for urine bladder carcinoma in the Swiss mice, incidences were comparable. Following rat data were re-examined (PWG) and the incidences (%, calculated on N=50 or N=60) were as follows: (ECHA: please see the table in the attachment: Metazachlor_Health effects_Belgium MSCA) <u>Discussion:</u> (i) The incidence of hepatocellular adenoma and carcinoma were slightly elevated above both study and in-house HCD level in the Wistar rat treated with Metazaclor (but it was within the RITA HCD database). In the SD rat, the incidence of hepatocellular carcinoma was also marginally high at the two highest doses tested, but the incidence was within HCD. The PWG considered that there might be a small treatment-related in the Wistar rats. The company further argued that Metazachlor was a CYP2B1-, 2B2-, 2C11- and 3A1-enzyme inductor similar to Phenobarbital (based upon an increase of mRNA levels after 3-7d treatment, rat strain and sex not reported) and based upon increased activities of CYP450 2B activities in a 14d study on female Wistar rats), and indicated therefore that the small increase of liver tumours in the Wistar-		

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		rats was of no relevance for the human. (ii) There was a marginally high C-cell carcinoma incidence in the top-dose male SD rats following administration of Metazachlor. However, dose-response was not evident and the incidence was within the HCD. Moreover, adenoma incidence was unaltered with the treatment. Therefore, it may be considered that the finding was a spurious event. Actually, lesions in the new histopathology assessment were performed according to new (better defined) diagnostic criteria, explaining why the carcinoma incidence in the old evaluation were no longer considered as malignant in the new evaluation. (iii) The incidence of thyroid follicular adenoma was increased in male SD rats at the top-dose. At the two highest doses, one animal was found with a follicular cell carcinoma. The incidence of both types of thyroid lesions were within the in-house HCD. In a mechanistic study, SD rats were exposed to Metazachlor in the diet during 28d. Thyroid changes (weight increase, slight hypertrophy/hyperplasia) were noted, alongside moderately increased TSH levels, however *without* decreased T4 or T3 levels. On the other hand, it was also demonstrated that this treatment was not a direct thyreotoxicant (PDA test). Overall, these mechanistic studies pointed towards an indirect MOA.		

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		(iv) In the Swiss mouse, Metazachlor induced a diffuse hyperplasia in the urinary bladder epithelium. However, focal hyperplasia incidence (more likely associated with pre-neoplastic events) remained unaffected. Likewise, no concomitant increase of neither transitional cell papilloma nor carcinoma was observed in the new PWG evaluation. Therefore, no carcinogenic action of the substance towards the urinary bladder was anticipated. Mechanistic studies confirmed the hyperplasia in the bladder (in both mice strains!), which was not caused by microcristallisation in the bladder lumen. (v) A slight increase of kidney cortical adenoma but no carcinoma was observed in the CD-1 mice. The incidence of benign tumours was slightly above HCD in the males. It was unclear what the MOA was for the increased pre-neoplastic tumours, as there was no indication of toxicity (no single-cell necrosis) or sustained regeneration (no mitotic figures). Therefore, the notifier concluded that the event was not treatment-related. However, the data only demonstrated that the observed increase was not explained by sustained proliferation, not that the finding was unrelated to treatment. (vi) A slight increase of hepatocellular adenoma was observed in the top-dose		

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		female CD-1 mice, however without concomitant increase in the number of hepatocellular carcinoma. Conclusion: The long-term treatment of rodents with Metazachlor was associated with: (i) a clear increase of hepatocellular tumours in the female Wistar rat. There was indirect evidence that the event was a phenobarbital-like event, associated with the induction of CYP 450. (ii) in the SD rat: a slight trend towards an increase - of hepatocellular carcinoma, without increase in the hepatocellular adenoma incidence, - of C-cell carcinoma, without increase in the C-cell adenoma incidence - of follicular adenoma, without meaningful increase of the carcinoma incidence (iii) in the CD-1 male mice an increase of the kidney cortical adenoma incidence, however without increase of the kidney carcinoma, and a trend towards an increase of hepatocellular adenoma, however without increased incidence of hepatocellular carcinoma.		

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPSAL ON METAZACHLOR

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		Except for the increased incidence of liver tumours in the female Wistar rats, and of kidney papillomas in the CD-1 male mice, all observed incidences were within in-house historical control data. RMS considered the mouse CD-1 kidney adenoma significant, however in the absence of frank malignant tumours, this remains doubtful. It is the opinion of BE that the only consistent and toxicologically meaningful increase was found in the female Wistar rat. In this case, notifier made a case that the tumour induction was associated with a phenobarbital-like MOA, which would be irrelevant for the human, however this is generally not acceptable as a sole explanation. Also the remark that only Wistar rats were affected, and no clear increase was seen in SD rat was not accepted, as one strain may be more sensitive than the other. Therefore, it is deemed justified to assign a classification as a Carc. Cat. 3 (Xn;R40) – Cat. 2 (H351) based upon the hepatocellular tumours in the female Wistar rats.		

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Mutagenicity

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
14/04/2010	Germany / Jan Averbecl / MSCA	Page 32 The German CA supports not to classify metazachlor for mutagenic hazard.	Thank you for these comments	

Toxicity to reproduction

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
14/04/2010	Germany / Jan Averbecl / MSCA	Page 50ff The German CA supports not to classify metazachlor for reproductive or developmental hazard.	Thank you for these comments	

Respiratory sensitisation

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
14/04/2010	Germany / Jan Averbecl / MSCA	Page 24 The German CA supports not to classify metazachlor for respiratory sensitizing hazard.	Thank you for these comments	

Other hazard classes

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
14/04/2010	Germany / Jan Averbecl / MSCA	The German CA supports the proposal for environmental classification and labelling of Metazachlor: according directive 67/548/EEC: N; R50/53 according regulation EC/1272/2008: Aquatic Acute 1 - H400	Thank you for these comments.	There is full agreement; the new results of the cited references further confirm the recommended classification.

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		Aquatic Chronic 1 - H410 M-factor: 100 The German CA provides as well additional new results for Metazachlor from recently published laboratory aquatic plant tests and mesocosms (3 references, see annex). Addition to chapter 7, point 7.1.1.3 Algae and aquatic plants The sensitivity of Lemna minor in the first new study (reference 1) is slightly higher than the relevant endpoint for M-factor 7-d ErC50 of Lemna gibba (2.8 µg/ L versus 7.1 µg/ L). This new result provides the same M-factor of 100. reference 1: Herbicide effects of metazachlor on duckweed (Lemna minor and Spirodela polyrhiza) in test systems with different trophic status and complexity (Müller et al. (2010): published at Journal of Environmental Science and Health, Part B (2010) 45, 95-101) The other two new studies provide additional information for effects of Metazachlor on higher tier aquatic systems. reference 2: Effects of the herbicide metazachlor on macrophytes and ecosystem function in	Thank you for submitting these new data. We have not been able to review the studies to assess their validity in the limited time available. Since they do not influence the proposed classification, we have briefly referred to them in a footnote but have not included any details in the report. We hope this is acceptable.	

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPSAL ON METAZACHLOR

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		freshwater pond and stream mesocosms (Mohr et al. (2007): published at Aquatic Toxicology 82 (2007) 73-84) reference 3: Response of plankton communities in freshwater pond and stream mesocosms to the herbicide metazachlor (Mohr et al. (2008): published at Environmental Pollution 152 (2008) 530-542)		
26/04/2010	Germany / Christiane Wiemann / BASF SE and Feinchemie Schwebda GmbH	CHAPTER 1 - Identity of substance and physical and chemical properties p. 6 Impurities: …..One impurity has been identified as being of possible toxicological relevance because it is classified for human health. This impurity, however is present < 0.01% and as such is significantly below the relevant concentration limits triggering classification….. Manufacturers' comment: Instead of 0.01% the number should read 0.05%. This number of 0.05% is given in Commission Directive 2009/155/EC of 30 November 2009 reflecting the situation for metazachlor. CHAPTER 3 – Classification and Labelling p.7 Proposed labelling CLP Regulation: Pictograms GHS07, GHS08, GHS09	We have amended this accordingly.	

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPSAL ON METAZACHLOR

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		Manufacturers' comment: The selected pictograms regarding toxicological hazards do not appropriately reflect the proposed hazard statements. Both H351 and H317 require the pictogram GHS07, while H400 and H410 both require GHS09. p.7 5.1 Toxicokinetics (absorption, metabolism, distribution and elimination) ….There are no available data on the absorption of pure metazachlor via the dermal route. However, the results of a human skin in vitro study conducted in one formulation identified an absorption value of 9%.... Manufacturers' comment The 9% absorption was determined on a spray diluted product (100-fold dilution), the formulation concentrate containing 50% metazachlor is considered to more appropriately reflect the dermal absorption of the active ingredient. Including the residues determined in the epidermis the potentially absorbed dose was less than 2% (please refer to the Draft Assessment Report). This value is also supported by other recent dermal absorption studies through human skin in vitro conducted with metazachlor product, which could be made available on request.	The selected pictograms are correct. Pictogram H351 requires GHS08 (health hazard) and not GHS07 (exclamation mark).	

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		CHAPTER 4 – Environmental Fate Properties p. 13 4.1.2.3 Simulation tests, Study 1, 3rd paragraph: ….. Various degradants were identified in water and sediment with BH 479-4 […..] and BH 479-6 […..] being the principle degradants at water maxima of 8.41 % AR and 8.87 % AR respectively in Millstream Pond. Manufacturers' comment: The water maximum of BH 479-6 should read 8.06 % AR instead of 8.87 % AR. p. 15 Overview: ….. The most significant degradants were BH479-4 and BH479-6 which were generally still increasing in concentration at study termination. … Manufacturers' comment: It is proposed to change the wording as follows: The most significant degradants were BH479-4 and BH479-6 which were partly still increasing in concentration at study termination. Considering all four water/sediment systems, the situation at study termination is the following: BH 479-4, water: increase in 3 out of 4	Thank you for pointing out this error – it has been corrected. OK – we have deleted the word 'generally' and added the words "in some (but not all) of the systems" to the end of the sentence.	

Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		systems BH 479-6, water: increase in 2 out of 4 systems BH 479-6, sediment: increase in 2 out of 4 systems BH 479-6, sediment: increase in none of the 4 systems In all other cases, the concentrations were constant or decreasing with changes of ≤ 0.1 %AR considered as constant. CHAPTER 5 - Human Health Hazard Assessment p.26 5.6.1.2 Mouse Table 5.7 Repeat dose studies: 28-day studies in mice Dose levels …. Corresponds to 0, 379, 891, 843 mg/kg body weight/day in females Manufacturers' comment The highest dose level must read 1843 mg/kg body weight/day	UK: Thank you we will amend the table as necessary.	
26/04/2010	Spain / Elina Valcare / MSCA	p 24 Summary and discussion of sensitisation The Spanish CA supports the proposed classification of Metazachlor as skin	Thank you for these comments	Noted.

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Date	Country/ Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
		sensitizer (R43: may cause sensitisation by skin contact) based on Directive 67/548/EEC and as Skin Sens.1 (H317: May cause an allergic skin reaction) based on CLP criteria.		

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPSAL ON METAZACHLOR

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPSAL ON METAZACHLOR

Annex 2 – Comments and response to comments on CLH proposal on Metazachlor – UK summary of additional information submitted by industry following the public consultation.

Information regarding the proposed mode of action of Metazachlor

Liver tumours

Industry has hypothesised that the metazachlor-induced liver tumours observed in female Wistar rats are caused by activation of the constitutive androstane receptor (CAR). Activation of CAR results in a pleiotropic response including the stimulation of cytochrome P450 (CYP) CYP2B forms and increased cell proliferation which ultimately leads to tumour formation. This mode of action is consistent with that established for phenobarbitone-induced liver tumours in mice and rats.

Additional evidence

Key event	Dose/concentration	Evidence
The effects of Metazachlor on CAR activation: a mechanism for the observed CYP2B induction (summary report) Wang, 2010	Rat liver tissue (strain not specified) from animals treated orally with 0 and 8000 ppm metazachlor 500 ppm phenobarbitone	Aim Study aimed at determining whether metazachlor induced expression of rat CYP2B was mediated by the activation of CAR Results Immunoblotting analysis indicated that the presence of CAR in the nucleus was higher for phenobarbitone and metazachlor treated rats compared to controls. Conclusion Metazachlor is capable of translocating and activating rat CAR *in vivo*
Induction of the CYP2B1 promoter by metazachlor-dependant CAR activation in primary cultures of rat hepatocytes Neuschafer-Rube and Puschel, 2010	Isolated rat hepatocytes from male Wistar rats metazachlor (0.1 – 100 μM) or 1 mM Phenobarbitone Real-time PCR and cell transfection assays used	Aim Study aimed to investigate whether 1) metazachlor induces CYP2B1 (a target gene of CAR) and 2) whether it does so using the CAR binding region within the promoter of CYP2B1 Results Part one: Does metazachlor induce CYP2B1 – a target gene of CAR? Metazachlor was shown to increase CYP2B1 expression 2-fold at 10 μM and 16-fold at 100 μM metazachlor. Using QPCR, phenobarbitone was able to induce CYP2B1 500-fold at 1 mM. Conclusion: metazachlor weakly activates CYP2B1 expression *Concern: Metazachlor was shown to be toxic to the hepatocytes, but Phenobarbitone was not.* Part 2: Does metazachlor activate CYP2B1 via the conserved CAR binding region within the promoter of CYP2B1 A luciferase reporter gene was constructed.

		Incubation with 1 mM Phenobarbitone led to a 2.5 fold increase in luciferase activity, whereas 100 µM metazachlor led to a 1.5 fold increase. Cytotoxicity was not investigated. Therefore it is not clear whether the low response seen with metazachlor at a concentration one order of magnitude less compared to phenobarbitone was due to cytotoxicity. No stimulation was observed when the binding element was missing, in fact, expression appeared to be reduced at the highest concentration (100 µM) although this may simply reflect cytotoxicity. Conclusion: Metazachlor appears to be a weak inducer of CAR.
S-phase response study in Wistar Rats administered metazachlor in the diet for 3, 7, 14 and 28 days Buesen et al, 2010	Female Wistar rats (10/group) Dosed in diet for either 3, 7, 14 or 28 days with 200 ppm or 8000 ppm equivalent to 13 mg/kg/day or 552-682 mg/kg/day metazachlor	Aim Study aimed at investigating whether administration of metazachlor results in increased cell proliferation in the liver of Wistar rats Results Liver weight was shown to significantly increase (> 10 %) after day 7. A significant increase in cell proliferation (measured by BrdU incorporation) was observed. The results indicated that administration of 8000 ppm led to an 8-fold increase in cell proliferation in the 3- day treated rats, a 12-fold increase in 7 day treated rats, a 15-fold increase in 14 day treated rats and, only, a 6-fold increase in 28-day treated rats. No significant increase in cell proliferation was observed in 200 ppm treated animals. Conclusion Metazachlor appears to stimulate cell proliferation in liver cells. It is unclear why the extent of the increase was less following 28-days than at other time points.

Tumours (adenomas and carcinomas) were observed in the liver of female Wistar rats and were considered treatment related by both the study pathologists and the PWG reviewers. Industry have hypothesised that these tumours were the result of a phenobarbitone-like response. In support of this argument industry have provided studies showing, both directly and indirectly, that metazachlor is a weak activator of CAR (which is consistent with the weak effects observed in the liver) and that administration of metazachlor results in proliferation of liver cells.

However, doubts for this mode of action are raised by the fact that a similar effect was not observed in mice, although they are the more sensitive species to phenobarbitone-induced liver tumours. Concern is also raised by the fact that metazachlor was shown to be toxic to isolated rat liver cells whereas phenobarbitone was not (Nuschafer-Rube).

There are no established criteria for regulatory acceptance of this mode of action, nor has agreement been reached that the effects of phenobarbitone are not relevant for humans. In previous discussions with industry we recommended they analyse the existing data in accordance with the IPCS framework for evaluating a mode of action for chemical carcinogenesis (Sonic-Mullin, Regulatory Toxicology and

Pharmacology 34, 146-152 (2001)) and the IPCS frame work for analysing the relevance of a cancer mode of action in humans (Boobis, Critical reviews in toxicology, 36, 781-792 (2006)). This tool allows clear and consistent documentation of the facts and brings transparency to the analysis and increases confidence in the conclusions reached. We feel that this analysis could be helpful to bring clarity to the issue and would suggest the rapporteur requests it.

A number of literature papers have also been submitted to support this postulated mode of action. These are referenced below and the RAC may wish to take them into consideration.

BASF_FCS_ 7 Whysner J, Ross PM, Williams GM (1996) Phenobarbital mechanistic data and risk assessment: enzyme induction, enhanced cell proliferation, and tumour promotion. Pharmacol.Ther. 71 (1-2) 153-191.

BASF_FCS_ 9 Hernandez JP, Mota LC, Huang W, Moore DD, Baldwin WS (2009) Sexually dimorphic regulation and induction of P450s by the constitutive androstane receptor (CAR). Toxicology 256 53-64.

BASF_FCS_ 10 Swales K, Negishi M (2004) CAR, Driving into the future. Minireview Molecular Endocrinology 18 (7) 1589-1598

BASF_FCS_ 11 Kodama S and Negishi M. (2006) Phenobarbital confers its divers effects by activating the orphan nuclear receptor CAR. Drug metabolism Reviews 38 (1) 75-87

BASF_FCS_ 15 Lake BG (2009). Species differences in the hepatic effects of inducers of CYP2B and CYP4A subfamily forms: relationship to rodent liver tumour formation. Xenobiotica 39, 582-596

Kidney tumours

S-phase response study in CD-1 mice administration in the diet for 7, 28 and 91 days Buesen et al, 2010 Hard, GC 2010	Male mice (10/group) Dosed in diet for either 3, 7, 14 or 28 days with 200, 700, 2500 and 4000 ppm BrdU incorporation	Aim Study aimed at investigating whether administration of metazachlor results in increased cell proliferation in the kidney of male mice Results No effect on kidney weight was observed. There was a statistically significant increase in cortical cell proliferation in both 28-day and 90-day treated animals from 200 ppm upwards. However, the increase was of low intensity (max 2.5 fold in the 2500 ppm group at 90-day) and the dose response was not clear. No histopathological effects were observed. Re-examination of the slides (Hard, 2010) indicated no differences of biological significance between controls and treated mice. Conclusion Metazachlor appears to slightly stimulate cell proliferation in kidney cells.

A small increase in adenoma incidence was observed in the kidney of male CD-1 mice. Re-examination of the slides suggested a mode of action based on sustained toxicity was unlikely. Investigation of cell proliferation in the kidney of mice administered metazachlor over 7, 28 and 90-days revealed a slight

increase in cell proliferation from day 28 onwards, which appeared to be treatment related, although the dose response was not clear.

Overall, the UK still considers that since the increase in adenomas in the kidney was dose related and the incidence in the mid and top dose was above the historical controls, that there is a weak carcinogenic response in the kidney of male CD-1 mice.

References

1. Li L and Wang H (2010) The effects of Metazachlor on CAR activation: a mechanism for the observed CYP2B induction, BASF DocID 2010/1056091
2. Neuschäfer-Rube F, Püschel GP (2010); Induction of the CYP2B1 promoter by Metazachlor-dependent CAR (NR1I3) activation in primary cultures of rat hepatocytes, BASF DocID 2010/1056090
3. Buesen R, Kaufmann W, Fabian E, Ravenzwaay B (2010) BAS 479 H (Metazachlor) S-phase response study in Wistar rats. Administration in the diet for 3, 7, 14 and 28 days. BASF DocID 2010/1056070
4. Buesen R. Amendment No. 1 to the report BAS 479 H (Metazachlor) S-Phase Response Study in Crl:CD1(ICR) mice; Administration in the diet for 7, 28 and 91 days, BASF DocID 2010/1055081
5. Hard GC (2010) Expert Re-examination of Quantitative Pathology Assessment of Proximal Tubule Cell Proliferation Activity in Kidneys of Mice Administered Metazachlor in the Diet for 7, 28, and 90 days, Final Report March 26, 2010, BASF DocID 2010/1054128

Additional Historical control data

Note: in some instances the time period for which these data have been gathered is larger than the recommended 5- year period.

Rats

Wistar

Study – dose range 0-8000 ppm – Krishnappa 2002

Liver

Source: RITA Data Base – Reference 1

Hepatocellular adenoma (Dates: Jan 94 – Feb 05)	
Females	Males
1.2 % (Range: 0 - 14%)	1.2 % (Range: 0 - 8%)
Hepatocellular carcinoma (Dates: Jan 94 – Feb 05)	
Females	Males
0.7% (Range: 0 – 4%)	1.3% (Range: 0 – 10%)

See also reference 15 for further historical control data in Wistar rats (Advinus) which are not summarised here.

Sprague- Dawley

Study – dose range 0-6000 ppm – Hunter 1983

Liver –Source: RITA database – (References 2 and 3) and ****Historical Histopathology data from control CD rat studies performed at Huntingdon Sciences -(Reference 4)***

Hepatocellular adenoma (Dates: Sept 83 – Oct 02)	
Females	Males
2.8% (range 0-15%)	2.5 % (range: 0- 12 %) *1.13 % (0-4 %) (Dates Mar 78 – Oct 84)
Hepatocellular carcinoma (Dates: Sept 83 – Oct 02)	
Females	Males
0.7% (range 0-6%)	2.7 % (range: 0- 8 %) *1.97 % (0-6%) (Dates Mar 78 – Oct 84)

*Thyroid - (References 5 and 6) and **Historical Histopathology data from control CD rat studies performed at Huntingdon Sciences -(Reference 4)*

Parafollicular tumours

Males
Parafollicular cells (i.e. C-cell) adenoma
13.2 % (range: 3.3-66 %) (Dates: Sept 83 – Oct 02) *0.63 % (range: 0 – 4 %) (Dates Mar 78 – Oct 84)
C-cell carcinoma
2.2 % (range: 0- 20 %) (Dates: Sept 83 – Oct 02) *6.93 % (range: 0 – 18.33 %) (Dates Mar 78 – Oct 84)

Follicular tumours

Males
Follicular cell adenoma
2.9 % (range: 0 - 8 %) (Dates: Sept 83 – Oct 02) *4.7 % (range: 0 – 13.33 %) (Dates Mar 78 – Oct 84)
Follicular cell carcinoma
1.5 % (range: 0 - 8 %) (Dates: Sept 83 – Oct 02) *1.18 % (range: 0-8 %) (Dates Mar 78 – Oct 84)

Leydig cells – (Reference 7)

Males
Leydig cell hyperplasia (focal)
5.9 % (range: 0- 22 %) (Dates: Sept 83 – Oct 02)
Leydig cell adenoma
4.2 % (range: 0- 12 %) (Dates: Sept 83 – Oct 02)

MICE

Swiss mice

Study –0- 4000 ppm – Kumar 2003

Historical control data in Swiss mice are available in references 12 and 16 (Advinus data) these are not summarised here.

CD-1 Mice

Study: 0- 2500 ppm – Barnard 1983

Source: Long term studies performed at Huntingdon Life Sciences

Liver (Reference 8)

Females
Hepatocellular adenoma
3.49 % (range: 0-9.8 %) (Dates: Jun-78 – Oct 84)

RITA database (May 90 – March 03) 7.9 % (Range: 0 – 21.7%) Reference 13
Hepatocellular carcinoma
1.14 % (range: 0- 4 %) Dates: Jun-78 – Oct -84 **RITA database (May 90 – March 03) 11.6 % (Range: 4 – 22%) – Reference 13**

Kidney (Reference 11)

Males
Cortical (renal tubule) adenoma/ papillary cystadenoma
Renal adenoma: 0.3 % (range: 0- 1.96 %) (Dates: Jun-78 – Oct 84) **RITA database (May 90 – March 03) 0.6% (Range: 0-4%) Reference 9**
Cortical (renal tubule) carcinoma
Renal Carcinoma: 0.27 % (range: 0- 3.85 %) (Dates: Jun-78 – Oct 84) **RITA database (May 90 – March 03) 0.2% (Range: 0 – 2%) Reference 10**

Lymphoreticular system – (Reference 11)

Original study findings **

Males
Lymphoblastic leukaemia
0 % (range: 0 %) (Dates: Jun-78 – Oct 84)
Lymphosarcoma
5.99 % (range: 0- 17.65 %) (Dates: Jun-78 – Oct 84)
Reticulum cell sarcoma
2.64 % (range: 0- 10.91 %) (Dates: Jun-78 – Oct 84)
Lymphoid leukaemia
1.17 % (range: 0- 3.85 %) (Dates: Jun-78 – Oct 84)
Myeloid Leukaemia
1.04 % (range: 0- 5.77 %) (Dates: Jun-78 – Oct 84)

** Only the historical control data for the original study pathologist's findings have been added.

Historical control data references

1. Anonymous (2009) Lesion-related Incidence Data - Rat Wistar, Liver Adenoma, hepatocellular, Carcinoma, hepatocellular. Report created: 05-Oct-2009, BASF DocID 2009/1110093
2. Anonymous (2008) Lesion-related Incidence Data - Rat SPRD, Liver: Adenoma, hepatocellular. Report created: 21-Jan-2008, BASF DocID 2008/1095200
3. Anonymous (2008) Lesion-related Incidence Data - Rat SPRD, Liver: Carcinoma, hepatocellular. Report created: 20-Feb-2008, BASF DocID 2008/1095199
4. Anonymous (2008b) Historical Histopathology Data Long term studies CD rats, Liver Tumours, Thyroid Tumours. Huntingdon Life Science issued February 11, 2008,
5. Anonymous (2008l) Lesion-related Incidence Data - Rat SPRD, Thyroid gland: Adenoma, C-cell. Report created: 21-Jan-2008,
6. Anonymous (2008m) Lesion-related Incidence Data - Rat SPRD, Thyroid gland: Adenocarcinoma, follicular cell, Adenoma, follicular cell, Carcinoma, C-cell. Report created: 20-Feb-2008,
7. Anonymous (2008n) Lesion-related Incidence Data - Rat SPRD, Testis: Adenoma, Leydig cell, Carcinoma, Leydig Cell, Hyperplasia, Leydig cell - Focal/multifocal, Hyperplasia, Leydig cell -Diffuse (severe). Report created: 11-Mar-2008,
8. Anonymous (2008e) Historical Histopathology Data Long term studies CD-1 Mice, Liver - Hepatocellular Tumours. Huntingdon Life Science issued March 10, 2008,
9. Anonymous (2008o) Lesion-related Incidence Data - Mouse CD-1, Kidney, Adenoma. Report created: 21-Jan-2008,
10. Anonymous (2008p) Lesion-related Incidence Data - Mouse CD-1, Kidney, Carcinoma. Report created: 20-Feb-2008,
11. Anonymous (2008) Historical Histopathology Data Long term studies CD-1 Mice, Lymphoreticular Tumours, Kidney Tumours, Urinary Bladder Tumours. Huntingdon Life Science issued February 26, 2008, BASF DocID 2008/1095170
12. Anonymous (2008g) Historical Data 40 Carcinogenicity Study in Swiss Albino Mice. 40.9: Histopathological (Non-neoplastic and Neoplastic) Findings of Combined Fate Mice. Kidneys, Urinary Bladder. Advinus Therapeutics HD-CARCI-M 40.9/Edition 6/2008
13. Anonymous (2008q) Lesion-related Incidence Data - Mouse CD-1, Liver, Adenoma, hepatocellular, Carcinoma, hepatocellular. Report created: 11-Mar-2008,
14. Anonymous (2008c) Historical Histopathology Data Long term studies CD rats, Testes - Interstitial Cell Tumours. Huntingdon Life Science issued March 7, 2008,
15. Anonymous (2008f) Historical Data 38 Combined Chronic Toxicity and Carcinogenicity Study in Rats. 38.16: Histopathological (Non-Neoplastic & Neoplastic) Findings of Combined Fates. Liver, Kidney, Urinary Bladder, Thyroids. Advinus Therapeutics HD-C.C.R 38/16/Edition 6/2008
16. Anonymous (2008h) Historical Data 40 Carcinogenicity Study in Swiss Albino Mice. 40.9: Histopathological (Neoplastic) Findings of Combined Fate Mice. Liver. Advinus Therapeutics HD-CARCI-M 40.9/Edition 6/2008 BASF DocID 2008/1095173

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPSAL ON METAZACHLOR

Annex 3 – Comments and responses to comments on CLH proposal on Metazachlor – Summary of additional information presented by industry following the public consultation.

BASF Reference	Study Title	Document ID	UK comment
Additional study reports presented following the public consultation			
BASF_FCS_8	Büsen R (2010) Amendment No. 1 to the report BAS 479 H (Metazachlor) Microcristallization in the urinary bladder and enzyme induction in liver and kidney of Wistar rats; Administration in the diet over two weeks, BASF Study No. 48C0219/99168, BASF DocID 2010/1053010,	2010/1053010	The Annex VI report has been updated to include reference to this amendment and the data for males included in the table. The study report is attached to the IUCLID.
BASF_FCS_12	Li L and Wang H (2010) The effects of Metazachlor on CAR activation: a mechanism for the observed CYP2B induction, BASF DocID 2010/1056091	2010/1056091	Summarised and referenced in Annex 2 to RCOM. The study report is attached to the IUCLID.
BASF_FCS_13	Neuschäfer-Rube F, Püschel GP (2010); Induction of the CYP2B1 promoter by Metazachlor-dependent CAR (NR1I3) activation in primary cultures of rat hepatocytes, BASF DocID 2010/1056090	2010/1056090	Summarised and referenced in Annex 2 to RCOM. The study report is attached to the IUCLID.
BASF_FCS_14	Buesen R, Kaufmann W, Fabian E, Ravenzwaay B (2010) BAS 479 H (Metazachlor) S-phase response study in Wistar rats. Administration in the diet for 3, 7, 14 and 28 days. BASF DocID 2010/1056070	2010/1056070	Summarised and referenced in Annex 2 to RCOM. The study report is attached to the IUCLID.

BASF_FCS_19	Buesen R (2010) BAS 479 H (Metazachlor) Mechanistic study in female Wistar rats after oral administration via the diet over 3 and 7 days, BASF DocID 2010/1043666	2010/1043666	The Annex VI report has been updated to include reference to this report. The study report is attached to the IUCLID.
BASF_FCS_20	Amendment No. 1 to the report BAS 479 H (Metazachlor) S-Phase Response Study in Crl:CD1(ICR) mice; Administration in the diet for 7, 28 and 91 days, BASF DocID 2010/1055081	2010/1055081	Summarised and referenced in Annex 2 to RCOM. The study report is attached to the IUCLID.
BASF_FCS_21	Hard GC (2010) Expert Re-examination of Quantitative Pathology Assessment of Proximal Tubule Cell Proliferation Activity in Kidneys of Mice Administered Metazachlor in the Diet for 7, 28, and 90 days, Final Report March 26, 2010, BASF DocID 2010/1054128	2010/1054128	Summarised and referenced in Annex 2 to RCOM. The study report is attached to the IUCLID.

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPSAL ON METAZACHLOR

BASF Reference	Study Title	Document ID	UK comment
Additional information referenced in comments submitted by industry during public consultation.			
BASF_FCS_1	Wiemann C and Kaufmann W (2010a) Metazachlor: Pathological Peer Review Process and Role of the Pathological Working Group, BASF DocID 2010/1052261	2010/1052261	Industry summary for RAC - no action taken by UK. Report is attached to the IUCLID.
BASF_FCS_2	Wiemann C and Kaufmann W (2010b) Metazachlor: Sequence of Tumour Incidences During Histopathological Peer Review and Pathology Working Group Conclusion, BASF DocID 2010/1052260	2010/1052260	Industry summary for RAC - no action taken by UK. Report is attached to the IUCLID.
BASF_FCS_3	Anonymous (2008) Lesion-related Incidence Data - Rat SPRD, Liver: Adenoma, hepatocellular. Report created: 21-Jan-2008, BASF DocID 2008/1095200	2008/1095200	Historical Control Data – summarised and referenced in Annex 2 to RCOM and attached to IUCLID.
BASF_FCS_4	Anonymous (2008) Lesion-related Incidence Data - Rat SPRD, Liver: Carcinoma, hepatocellular. Report created: 20-Feb-2008, BASF DocID 2008/1095199	2008/1095199	Historical Control Data – summarised and referenced in Annex 2 to RCOM and attached to IUCLID.
BASF_FCS_5	Anonymous (2009) Lesion-related Incidence Data - Rat Wistar, Liver Adenoma, hepatocellular, Carcinoma, hepatocellular. Report created: 05-Oct-2009, BASF DocID 2009/1110093	2009/1110093	Historical Control Data – summarised and referenced in Annex 2 to RCOM and attached to IUCLID.
BASF_FCS_6	Wiemann C and Kaufmann H (2010) Metazachlor: Manufacturers' position on Annex VI report evaluation of rat liver carcinogenicity and mode of action, BASF DocID 2010/1054117	2010/1054117	Industry summary for RAC - no action taken by UK. Report is attached to the IUCLID.
BASF_FCS_7	Whysner J, Ross PM, Williams GM (1996) Phenobarbital mechanistic data and risk assessment: enzyme induction, enhanced cell proliferation, and tumour promotion. Pharmacol.Ther. 71 (1-2) 153-191.		Literature paper - further information for RAC to consider, referenced in Annex 2 but not summarised.
BASF_FCS_9	Hernandez JP, Mota LC, Huang W, Moore DD, Baldwin WS (2009) Sexually dimorphic regulation and induction of P450s by the constitutive androstane receptor (CAR). Toxicology 256 53-64.		Literature paper - further information for RAC to consider, referenced in Annex 2 but not summarised.

BASF_FCS_10	Swales K, Negishi M (2004) CAR, Driving into the future. Minireview Molecular Endocrinology 18 (7) 1589-1598		Literature paper - further information for RAC to consider, referenced in Annex 2 but not summarised.
BASF_FCS_11	Kodama S and Negishi M. (2006) Phenobarbital confers its divers effects by activating the orphan nuclear receptor CAR. Drug metabolism Reviews 38 (1) 75-87		Literature paper - further information for RAC to consider, referenced in Annex 2 but not summarised.
BASF_FCS_15	Lake BG (2009). Species differences in the hepatic effects of inducers of CYP2B and CYP4A subfamily forms: relationship to rodent liver tumour formation. Xenobiotica 39, 582-596		Literature paper - further information for RAC to consider, referenced in Annex 2 but not summarised.
BASF_FCS_16	Wiemann C and Kaufmann W (2009) Metazachlor - Explanation on open points raised by RMS United Kingdom in the draft Annex VI Report: Proposal for harmonised classification and labelling including corrected tables and revised historical control data	2009/1109594	Industry summary for RAC - no action taken by UK. Report is attached to the IUCLID.
BASF_FCS_17	Anonymous (2008) Historical Histopathology Data Long term studies CD-1 Mice, Lymphoreticular Tumours, Kidney Tumours, Urinary Bladder Tumours. Huntingdon Life Science issued February 26, 2008, BASF DocID 2008/1095170	2008/1095170	Historical Control Data – summarised and referenced in Annex 2 to RCOM and attached to IUCLID.
BASF_FCS_18	Wiemann C and Kaufmann W (2010) Metazachlor: Manufacturers' position on Annex VI report evaluation of treatment relationship of kidney tumour formation in male CD1 mice, BASF DocID 2010/1054118	2010/1054118	Industry summary for RAC - no action taken by UK. Report is attached to the IUCLID.
BASF_FCS_22	Anonymous (2008a) To whom it may concern: BASF, Makhteshim-Agan and Feinchemie position on proposed R40 classification of Metazachlor - detailed assessment	2008/1078374	Industry position on R40. Was submitted to CA during drafting of proposal and was taken into consideration. No submitted to the RAC for further information.

BASF_FCS_23	Wall HG (2008a) Pathology Working Group (PWG) Review of the Carcinogenic Potential of Metazachlor: Liver and Thyroid Gland of Sprague-Dawley and Wistar Rats. HRC Study No BSF 326/8226/2 reissued 11 May 1983, HRC Study No. BSF 340/82449/2 reissued 9 May 1983, Rallis Study No. TOXI-1328 C:C_R; 27 May 2002 - Pathology Working Group Report. Experimental Pathology Laboratories (EPL) Study 717-009, Final report: September 16, 2008,	2008/1070697	Was already referenced in Appendix 1 to the CLH report. It was also attached to the IUCLID.
BASF_FCS_24	Wall HG (2008b) Pathology Working Group (PWG) Review of the Carcinogenic Potential of Metazachlor: Interstitial Cell (Leydig) Cell Tumours of Sprague-Dawley Rats. HRC Study No BSF 326/8226/2 reissued 11 May 1983 - Pathology Working Group Report. Experimental Pathology Laboratories (EPL) Study 717-009, Final report: September 16, 2008,	2008/1070691	Was already referenced in Appendix 1 to the CLH report. It was also attached to the IUCLID.
BASF_FCS_25	Wall HG (2008c) Pathology Working Group (PWG) Review of the Carcinogenic Potential of Metazachlor: Proliferative Lesions in the Urinary Bladder in Swiss Albino Mice. Rallis Study No. 1329 (24 April, 2003) - Pathology Working Group Report. Experimental Pathology Laboratories (EPL) Study 717-009, Final report: September 16, 2008,	2008/1070699	Was already referenced in Appendix 1 to the CLH report. It was also attached to the IUCLID.
BASF_FCS_26	Wall HG (2008d) Pathology Working Group (PWG) Review of the Carcinogenic Potential of Metazachlor: Lymphoreticular Tumours in Male CD-1 (Charles River) Mice. HRC Study No BSF 327/82389 (27 April, 1983) - Pathology Working Group Report. Experimental Pathology Laboratories (EPL) Study 717-009, Final report: September 16, 2008,	2008/1070700	Was already referenced in Appendix 1 to the CLH report. It was also attached to the IUCLID.
BASF_FCS_27	Wall HG (2008e) Pathology Working Group (PWG) Review of the Carcinogenic Potential of Metazachlor: Kidney Tumours in Male Mice. HRC Study No BSF 327/82389 (27 April, 1983) and Rallis Study No. 1329 (24 April, 2003) - Pathology Working Group Report. Experimental Pathology Laboratories (EPL) Study 717-009, Final report: September 16, 2008,	2008/1070692	Was already referenced in Appendix 1 to the CLH report. It was also attached to the IUCLID.

BASF_FCS_28	Wall HG (2008f) Pathology Working Group (PWG) Review of the Carcinogenic Potential of Metazachlor: Liver Tumours of CD-1 (Charles River) Female Mice. HRC Study No BSF 327/82389 issued 27 April 1983 - Pathology Working Group Report. Experimental Pathology Laboratories (EPL) Study 717-009, Final report: September 16, 2008,	2008/1070698	Was already referenced in Appendix 1 to the CLH report. It was also attached to the IUCLID.
BASF_FCS_29	Anonymous (2008b) Historical Histopathology Data Long term studies CD rats, Liver Tumours, Thyroid Tumours. Huntingdon Life Science issued February 11, 2008,	2008/1095179	Historical Control Data – summarised and referenced in Annex 2 to RCOM and attached to IUCLID.
BASF_FCS_30	Anonymous (2008c) Historical Histopathology Data Long term studies CD rats, Testes - Interstitial Cell Tumours. Huntingdon Life Science issued March 7, 2008,	2008/1095180	Historical Control Data - referenced in Annex 2 to RCOM and attached to IUCLID.
BASF_FCS_31	Anonymous (2008e) Historical Histopathology Data Long term studies CD-1 Mice, Liver - Hepatocellular Tumours. Huntingdon Life Science issued March 10, 2008,	2008/1095169	Historical Control Data – summarised and referenced in Annex 2 to RCOM and attached to IUCLID.
BASF_FCS_32	Anonymous (2008f) Historical Data 38 Combined Chronic Toxicity and Carcinogenicity Study in Rats. 38.16: Histopathological (Non-Neoplastic & Neoplastic) Findings of Combined Fates. Liver, Kidney, Urinary Bladder, Thyroids. Advinus Therapeutics HD-C.C.R 38/16/Edition 6/2008	2008/1095172	Historical Control Data - referenced in Annex 2 to RCOM and attached to IUCLID.
BASF_FCS_33	Anonymous (2008g) Historical Data 40 Carcinogenicity Study in Swiss Albino Mice. 40.9: Histopathological (Non-neoplastic and Neoplastic) Findings of Combined Fate Mice. Kidneys, Urinary Bladder. Advinus Therapeutics HD-CARCI-M 40.9/Edition 6/2008	2008/1095174	Historical Control Data - referenced in Annex 2 to RCOM and attached to IUCLID.
BASF_FCS_34	Anonymous (2008h) Historical Data 40 Carcinogenicity Study in Swiss Albino Mice. 40.9: Histopathological (Neoplastic) Findings of Combined Fate Mice. Liver. Advinus Therapeutics HD-CARCI-M 40.9/Edition 6/2008 BASF DocID 2008/1095173	2008/1095173	Historical Control Data - referenced in Annex 2 to RCOM and attached to IUCLID.
BASF_FCS_35	Anonymous (2008l) Lesion-related Incidence Data - Rat SPRD, Thyroid gland: Adenoma, C-cell. Report created: 21-Jan-2008,	2008/1095195	Historical Control Data – summarised and referenced in Annex 2 to RCOM and attached to IUCLID.

BASF_FCS_36	Anonymous (2008m) Lesion-related Incidence Data - Rat SPRD, Thyroid gland: Adenocarcinoma, follicular cell, Adenoma, follicular cell, Carcinoma, C-cell. Report created: 20-Feb-2008,	2008/1095194	Historical Control Data – summarised and referenced in Annex 2 to RCOM and attached to IUCLID.
BASF_FCS_37	Anonymous (2008n) Lesion-related Incidence Data - Rat SPRD, Testis: Adenoma, Leydig cell, Carcinoma, Leydig Cell, Hyperplasia, Leydig cell - Focal/multifocal, Hyperplasia, Leydig cell -Diffuse (severe). Report created: 11-Mar-2008,	2008/1095196	Historical Control Data – summarised and referenced in Annex 2 to RCOM and attached to IUCLID.
BASF_FCS_38	Anonymous (2008o) Lesion-related Incidence Data - Mouse CD-1, Kidney, Adenoma. Report created: 21-Jan-2008,	2008/1095190	Historical Control Data – summarised and referenced in Annex 2 to RCOM and attached to IUCLID.
BASF_FCS_39	Anonymous (2008p) Lesion-related Incidence Data - Mouse CD-1, Kidney, Carcinoma. Report created: 20-Feb-2008,	2008/1095201	Historical Control Data – summarised and referenced in Annex 2 to RCOM and attached to IUCLID.
BASF_FCS_40	Anonymous (2008q) Lesion-related Incidence Data - Mouse CD-1, Liver, Adenoma, hepatocellular, Carcinoma, hepatocellular. Report created: 11-Mar-2008,	2008/1095191	Historical Control Data – summarised and referenced in Annex 2 to RCOM and attached to IUCLID.