Annex VI Report

PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

Substance Name: Metazachlor

EC Number: 266-583-0

CAS Number: 67129-08-2

Submitted by: UK REACH Competent Authority
Chemicals Regulations Directorate
Health and Safety Executive
Bootle
Merseyside
L20 7HS
United Kingdom

E-mail: Sarah.dutton@hse.gsi.gov.uk

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BACKGROUND

Metazachlor is a chloroacetanilide herbicide used on oilseed rape. In 2008 it was approved for Annex I listing as a 3A Review compound under Council Directive 91/414/EEC, with the UK as Rapporteur Member State. In accordance with Article 36(2) of the CLP Regulation, metazachlor should now be considered for harmonised classification and labelling. Therefore, this proposal considers all human health and environmental endpoints. This Annex VI dossier presents a classification and labelling proposal based mainly on the information presented in the assessment of metazachlor under Directive 91/414/EEC. This assessment, referred to as the Pesticide Assessment Report in this document, was based on two full data packages submitted by two separate companies and is attached to the IUCLID5 dossier.

Metazachlor is not currently listed in Annex VI of Regulation EC 1272/2008 (CLP Regulation). Following evaluation of the data this proposal seeks to propose classifications for skin sensitisation, carcinogenicity and the environment.

PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

Substance name: Metazachlor

EC number: 266-583-0

CAS number: 67129-08-2

Registration number(s): Active substances used in plant protection products and included in Annex I to Council Directive 91/414/EEC are regarded as registered under REACH (Article 15(1) of REACH). No formal registration numbers are assigned to these substances.

Purity: The minimum purity of metazachlor produced by either company is 95 %.

Impurities: The manufacturers have requested that all impurities remain confidential. According to the present specifications of industrially-produced metazachlor from the two companies, the majority of impurities identified are present at <1 % (typically < 0.5%) with only one impurity present in each company's formulation at > 1 %. 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. Given the low levels it is considered to have no significant impact on the hazardous properties of metazachlor itself. None of the other impurities are of toxicological or environmental concern.

Proposed classification based on Directive 67/548/EEC:

Class of Danger	Xn: Harmful N: Dangerous for the environment
R-Phrases	R43: May cause sensitisation by skin contact R40 (Carc. Cat 3): Limited evidence of a carcinogenic effect R50-53: Very toxic to aquatic organisms

Proposed classification based on CLP Criteria:

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

Proposed labelling:

Directive 67/548/EEC:
Xn
R40, R43, R50-53
S: 36-37-60-61

CLP Regulation:
Signal Word: Warning
Pictograms: GHS07 GHS 08 GHS09
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

Proposed specific concentration limits (if any):

Classification of the preparation		
N, R50-53	N, R51-53	R52-53
Cn ≥ 0.25 %	0.025 % ≤ Cn <0.25 %	0.0025 % ≤ Cn <0.025 %

Where Cn is the concentration of metazachlor in the preparation.

Under CLP M factor 100 based on 0.001 <L(E)C_{50} ≤0.01 mg/l.

Proposed notes (if any): None

JUSTIFICATION

1 IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES

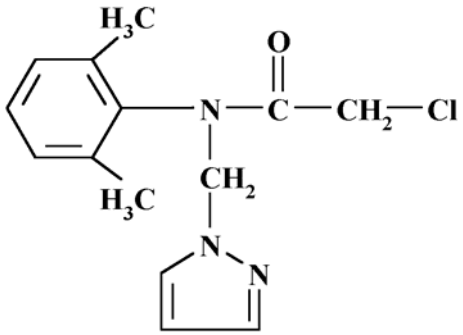
1.1 Name and other identifiers of the substance

Chemical Name: Metazachlor
EC Number: 266-583-0
CAS Number: 67129-08-2
IUPAC Name: 2-chloro-N-(2,6-dimethylphenyl)-N-(1H-pyrazol-1-ylmethyl)acetamide

1.2 Composition of the substance

Chemical Name: Metazachlor
EC Number: 266-583-0
CAS Number: 67129-08-2
IUPAC Name: 2-chloro-N-(2,6-dimethylphenyl)-N-(1H-pyrazol-1-ylmethyl)acetamide
Molecular Formula: $C_{14}H_{16}ClN_3O$

Structural Formula:



Molecular Weight:	277.8
Typical Concentration (% w/w)	> 95% for both companies (only one impurity in each formulation is present at > 1 % in the material placed on the market)
Concentration range (% w/w)	Confidential information

1.3 Physico-Chemical properties

The physico-chemical properties of metazachlor have been well investigated, as summarised in the Pesticide Assessment Report attached to the IUCLID 5 dossier. Some of the key information is provided in the table below. In all the studies below the purity of the test substance was ≥ 96.6 %. No classification is justified.

Table 1 Summary of physico-chemical properties

REACH ref Annex, §	Property	Value	Reference and method
VII, 7.1	Physical state at 20 C and 101.3 KPa	Colourless crystalline solid with no odour.	Daum, 1999c in reference 1
VII, 7.2	Melting / freezing point	82.5 ^{o}C	Kastel, 1999 in reference 1 EU A1
VII, 7.3	Boiling point	The substance decomposes at 220 ^{o}C before boiling.	Daum, 1999c in reference 1 EU A2 - DSC
VII, 7.4	Relative density	1.303	Kastel, 1998 in reference 1 EU A3
VII, 7.5	Vapour pressure	9.5 x 10-5 Pa at 20^{o}C 0.22 x 10-5 Pa at 25^{o}C	Guckel, 1991 in reference 1 Evaporation rate method equivalent to EU A4 "Effusion method: by loss of weight.
VII, 7.6	Surface tension	59.0 mN/m (2 % w/w concentration) 60.2 mN/m (0.5 % w/w concentration) 62.8 mN/m (0.1% and 1% concentrations)	Kastel, 1998 in reference 1 EU A5 Kastel, 1999 in reference 1 EU A5

VII, 7.7	Water solubility	450 mg/l at pH 7 and 20°C 630 mg/l at pH 7 and 25 °C	Redeker, 1991 in reference 1 EU A6 Schneider 1998 in reference 1 OECD 105 (Shake flask)
VII, 7.8	Partition coefficient n-octanol/water (log value)	2.49 at 21°C and pH 7	Daum, 1998a in reference 1 OEDC 117 HPLC Method
VII, 7.10	Flammability	The test substance did not burn.	Loffler, 1999 in reference 1 EU A10
VII, 7.12	Self-ignition temperature	No self ignition was observed at temperatures up to 400°C	Loffler, 1999 in reference 1 EU A16
VII, 7.11	Explosive properties	Not explosive (Tested by thermal sensitivity, mechanical sensitivity-shock and friction.)	De Ryckel 2001 in reference 1 EU A14
VII, 7.13	Oxidising properties	Assessment of the structure indicates that the substance does not have the potential to be oxidising.	Justification in reference 1
IX, 7.16	Dissociation constant	At 20°C and a concentration of 16 mg/l no pKa determination could be made. From the structure of Metazachlor it is assumed that it will not dissociate in water to from ionic species.	Daum. 1998b in reference 1 OECD 112

2 MANUFACTURE AND USES

Metazachlor is manufactured and placed on the market as an herbicide used on oil seed rape.

3 CLASSIFICATION AND LABELLING

3.1 Classification in Annex VI of the CLP Regulation

The substance is not listed in Annex VI of the CLP Regulation.

3.2 Self Classification(s)

The following classification is applied by industry:

Class of Danger	N: Dangerous to the Environment Xi: Irritant
R-Phrases	R43: May cause skin sensitisation by skin contact R50/53: Very toxic to aquatic organisms

4 ENVIRONMENTAL FATE PROPERTIES

A detailed summary of the available studies has been reviewed and their robustness determined under Directive 91/414/EEC and is provided in the Pesticide Assessment Report (DAR) which is attached to the IUCLID 5 dossier. The key information pertinent to determining a classification position is presented below.

Studies[1] described in this section were undertaken with metazachlor (parent active substance) and the following aquatic degradants; BH 479-4 [N-(2,6-dimethylphenyl)-N-(1H-pyrazol-1-ylmethyl)oxalamide]; and BH 479-6 [N-(2,6-dimethylphenyl)-N-(1H-pyrazol-1-ylmethyl)acetamide].

4.1 Degradation

4.1.1 Stability

Hydrolysis
Two hydrolysis studies are available using metazachlor following OECD guideline 111.

The first study using phenyl-U-^{14}C radiolabelled metazachlor was run at pH 4, 7 and 9 at 50^{o}C, and at pH 4, 5, 7 and 9 at 25^{o}C (Class. T (2002) in reference 11). Only at 50^{o}C and pH 4 and 9 was hydrolysis observed. At 25^{o}C and all pH values, assuming first order degradation, the DT_{50} values are considered to be >100 days.

The second study using unlabelled metazachlor measured hydrolysis at pH4, 7 and 9 at 50^{o}C, 60^{o}C and 70^{o}C (Schneider E (1998) and Stecher A(2003) in reference 11). Assuming first order degradation, the estimated DT_{50} values at 20^{o}C are considered to be 629 days at pH 4, 1238 days at pH7, and 397 days at pH9.

Overall, the two studies show that metazachlor is hydrolytically stable under environmentally relevant pH and temperature conditions.

Aqueous photolysis
An aquatic photolysis study is not available.

The molar decadic absorption coefficient of metazachlor for wavelengths between 290 and 300 nm, and above 300 nm, is <10 mol^{-1} cm^{-1} (Schneider E (1998) and Sarafin R (1991) in reference 11). This means no light energy was absorbed over wavelengths associated with natural sunlight. On this basis, direct aqueous photolysis in the environment is not considered to occur.

4.1.2 Biodegradation

4.1.2.1 Biodegradation estimation

As measured data are available estimation is not relevant for this dossier.

[1] Studies included in this section refer primarily to aquatic fate. Additional studies are available for fate properties in soil. These are not relevant for the purpose of classification and labelling and are therefore not included.

4.1.2.2 Screening tests

In a respirometric ready biodegradation study (Strotmann, U (1991) in reference 11) following EEC 79/831 using unlabelled metazachlor, 0 % degradation was achieved by day 28. Therefore, metazachlor is considered not readily biodegradable under the conditions of the test.

4.1.2.3 Simulation tests

Two aerobic water/sediment studies assessing the fate of metazachlor are available following SETAC guidelines, EPA guideline 162-4 and the BBA IV 5-1 guideline.

Study 1
Following SETAC guidelines, EPA guideline 162-4, d BBA IV 5-1 and draft OECD 308 guidelines, the study used two UK water/sediment systems called 'Millstream Pond' and 'Swiss Lake' (Schnoeder, F (2003) in reference 11). Using ^{14}C-phenyl radiolabelled metazachlor, flasks were incubated in the dark at 20 ±1 ^{o}C for up to 100 days.

Applied Radioactivity (AR) concentrations in water decreased from day 1 and by day 32 accounted for 23.72 % AR in Millstream Pond and 41.04 % AR in Swiss Lake. This decrease coincided with an increase in extractable sediment AR (by day 32, 19.65 % AR in Millstream Pond and 13.17 % AR in Swiss Lake) and non-extractable sediment AR (by day 32, 56.70 % AR in Millstream Pond and 44.41 % AR in Swiss Lake). Carbon dioxide was not detected until day 99 when 0.95 % AR and 0.72 % AR was determined in Millstream Pond and Swiss Lake respectively.

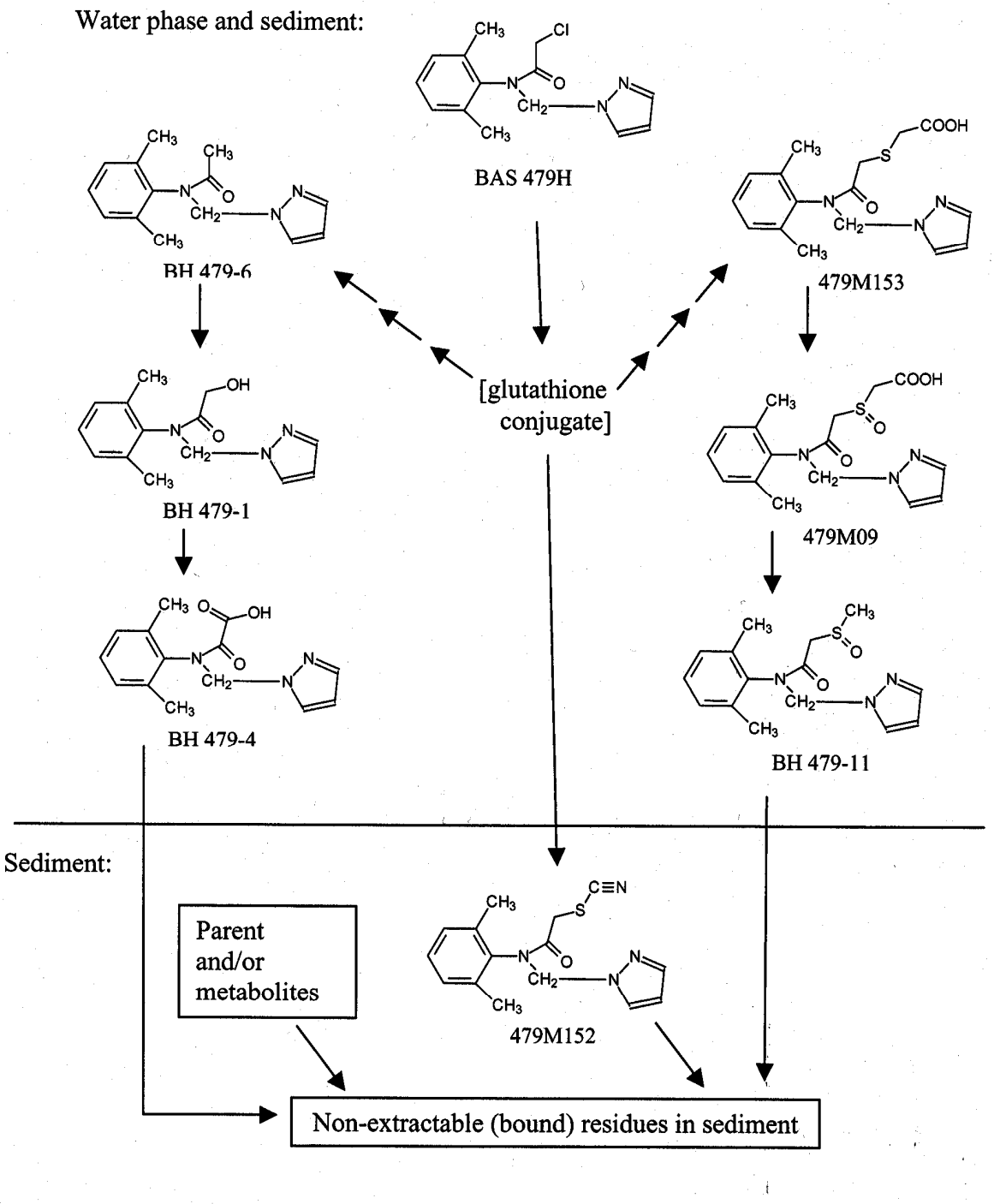
In both systems, metazachlor was observed to decrease in the water phase and the sediment phase. Various degradants were identified in water and sediment with BH 479-4 [N-(2,6-dimethylphenyl)-N-(1H-pyrazol-1-ylmethyl)oxalamide] and BH 479-6 [N-(2,6-dimethylphenyl)-N-(1H-pyrazol-1-ylmethyl)acetamide] being the principle degradants at water maxima of 8.41 % AR and 8.87 % AR respectively in Millstream Pond .

During the studies, radioactivity in water continuously decreased with an increase in radioactivity in sediment. When partitioned to sediment non-extractable sediment residues were mostly formed and mineralisation was negligible. The DT_{50} values for metazachlor dissipation in water were 6.5 days and 11.2 days for Millstream Pond and Swiss Lake. Based on dissipation from the whole system, the metazachlor DT_{50} values for the Millstream Pond and Swiss Lake are estimated to be 13.4 and 23.0 days. Based on degradation, DT_{50} values for metazachlor in water for the Millstream Pond and Swiss Lake are estimated to be 144 and 133 days.

Levels of degradants BH 479-4 and BH 479-6 were generally still increasing at study termination. At day 99 in Mill Pond the respective concentrations were: 8.41 % AR in water and 2.79 % AR in sediment; and 8.06 % AR in water and 7.96 % AR in sediment. At day 99 in Swiss Lake the respective concentrations were: 7.33 % AR in water and 1.58 % AR in sediment; and 7.91 % AR in water and 5.07 % AR in sediment. While there is uncertainty regarding partitioning rate constants for the degradant BH 479-6, $DT_{50,}$ estimates based on degradation in water were calculated for Millstream Pond (45.4 days) and Swiss Lake (27.1 days). No DT_{50} estimates are available for BH 479-4.

Based on this study a proposed degradation pathway for metazachlor in aerobic water/sediment systems is presented in figure 1.

Figure 1 - A proposed degradation pathway for metazachlor



BAS 479H = parent metazachlor

Study 2
Following BBA IV 5-1 guidelines, the study used two German water/sediment systems called 'Schaephysen pond' and 'Rückhaltebecken / Selbeckerbach river reservoir' (Feser-Zugner, W (200 and 2003) in reference 11). Using phenyl U-^{14}C radiolabelled metazachlor, flasks were incubated in the dark at 20 ±1^{o}C for up to 121 days.

Applied radioactivity concentrations in water decreased from the start of the test to 21.5 % AR and 46.8 %AR in Schaephysen and Rückhaltebecken / Selbeckerbach respectively. During this time, AR in sediment increased with the fraction bound in non-extractable residues also increasing with time. By day 30, 73.6 % AR was observed in Schaephysen sediment with 28.3 % AR in non-extractable sediment residues. At this time, 52.4 % AR was observed in Rückhaltebecken / Selbeckerbach sediment with 12.5 % AR in non-extractable sediment residues. Carbon dioxide was undetected until day 30 in Schaephysen (0.2 % AR) and day 15 in Rückhaltebecken / Selbeckerbach (0.1 % AR). The highest CO_2 measurement was 1.3 % AR on day 121 in Rückhaltebecken / Selbeckerbach.

Degradants BH 479-4 (maximum 10.9 % AR) and BH 479-6 (maximum 6.95 % AR) were identified with various additional degradants (BH 479-8, BH 479-10 and BH 479-11) generally below 1 % AR. Due to analytical limitations, quantification of components is not considered reliable. Metazachlor results are include here as supporting information. Based on dissipation from the whole system, the metazachlor DT_{50} values for the Schaephysen and Rückhaltebecken / Selbeckerbach are estimated to be 16.1 and 27.8 days. Based on mineralisation, DT_{50} values for metazachlor in water for the Schaephysen and Rückhaltebecken / Selbeckerbach are estimated to be 48.8 and 384 days.

Overview
In the four systems, mineralisation to carbon dioxide was minimal and formation of non-extractable sediment residues was the most significant route of dissipation. The most significant degradants were BH479-4 and BH479-6 which were generally still increasing in concentration at study termination. The studies indicate that metazachlor does not undergo significant mineralisation within 28 days.

4.1.3 Summary and discussion of persistence

Metazachlor is considered hydrolytically stable under environmentally relevant pH and temperature conditions. It is not considered to undergo photodegradation in the environment. On the basis of a ready biodegradation study, it is not considered readily biodegradable.

The fate of metazachlor in four aerobic water/sediment systems indicates that mineralisation to carbon dioxide is slow. The relatively short dissipation half life in water and low degradant concentrations in water indicate that metazachlor is partitioned to the sediment phase where degradation and formation of non-extractable sediment residues occurs. The estimated DT_{50} values indicate that metazachlor is not rapidly degradable and does not undergo significant mineralisation within 28 days (>70%). This means metazachlor is considered not readily biodegradable for the purpose of classification and labelling.

4.2 Environmental distribution

4.2.1 Adsorption / desorption

Various adsorption and desorption studies are available for metazachlor and aquatic degradants[2]. In general, due to low adsorption and desorption screening results, desorption values were not able to be determined.

Study 1- metazachlor
Using ^{14}C radiolabelled metazachlor adsorption and desorption coefficients were determined for three soils (loam, loamy sand, and sand) (Redeker, J (1979) in reference 11). While the study was not performed according to GLP compliant guidelines, it is considered acceptable as the experimental design was satisfactorily documented and the results are considered reliable. The K_{oc} adsorption constant range was 72.5 - 83.4 ml/g (sand to loam soils). The adsorption to two soils was too weak to allow a desorption constant to be achieved. For the loamy sand a desorption constant of 110.9 ml/g was calculated.

Study 2- metazachlor
Following OECD Guideline 106 and using phenyl-^{14}C radiolabelled metazachlor, adsorption coefficients were determined for four soils (sand, loamy sand, sandy loam and sandy silt) (Theis, M (2000 in reference 11). The K_{oc} adsorption constant range was 29.2 ml/g for the sandy silt to 73.1 ml/g for the loamy sand.

Study 3- metazachlor
Following OECD Guideline 106 and using ^{14}C radiolabelled metazachlor, adsorption and desorption coefficients were determined for four soils (silty sand, loamy silt, loamy sand and sandy loam) (Müller, J(2002) in reference 11). The K_{oc} adsorption constant range was 53.8 ml/g for the sandy loam to 97 ml/g for the silty sand. The K_{oc} desorption constant range was 94.1 ml/g for the loamy silt to 209.5 ml/g for the silty sand.

Study 4- metazachlor
Adsorption coefficients were determined using a commercially available formulation containing metazachlor (Allen, R and Walker, A (1987) in reference 11). The study was not performed according to GLP compliant guidelines, but the experimental design was satisfactorily documented and the study is considered reliable and therefore acceptable. The study comprised eighteen soils of various composition. The K_{oc} adsorption constant range was 78.6 ml/g (predominantly sand) to 220 ml/g (predominantly clay).

Study 5, 6 and 7 – aquatic degradant BH479-4
Adsorption coefficients were also determined for the major aquatic and soil degradant BH 479-4. The studies followed OECD Guideline 106, EPA guideline 163-1 and SETAC guidelines.

The first study used a phenyl-U-^{14}C radiolabel and four soils (sand, sand/loamy sand, sandy loam and a second sandy loam) (Seher, A (1998) in reference 11). The K_{oc} adsorption constant range was 1 – 2 ml/g.

The second study used four soils (sand, loamy sand, sandy loam and a second sandy loam) (Keller, W (1991) in reference 11). The K_{oc} adsorption constant range was 9 – 94 ml/g.

[2] Studies included in this section refer primarily to metazachlor and aquatic degradants. Additional studies are available for soil degradants. These are not relevant for the purpose of classification and labelling and are therefore not included.

The third study used three soils (sand, and two silty sand soils) (Heintze, A (2001) in reference 11). The K_{oc} adsorption constant range was 9.1 - 29.6 ml/g.

Study 8– aquatic degradant BH479-6
Adsorption coefficients were also determined for the major aquatic degradant BH 479-6 (Seher, A (1998) in reference 11). The study used a phenyl-U-^{14}C radiolabel, four soils and followed OECD Guideline 106, and EPA guideline 163-1. The K_{oc} adsorption constant range was 44 - 62 ml/g. The K_{oc} desorption constant range was 206 to 490 ml/g.

Overview
Metazachlor is a moderately water soluble substance and is not considered to dissociate in water (Daum (1998) and Schneider, E (1998) in reference 1). Therefore adsorption to soil is not considered to be affected by soil pH. Using all soil results, the metazachlor mean and median K_{oc} values based on the above studies are 114.4 ml/g and 110 ml/g. This indicates metazachlor is unlikely to adsorb strongly to solid matrices and is likely to be mobile.

Using all soil results, the mean and median K_{oc} values for the aquatic degradant BH 479-04 are 23.05 ml/g and 9.1 ml/g. For the aquatic degradant BH 479-06, the mean and median K_{oc} values are 53.7 ml/g and 54.5 ml/g. These values indicate that both degradants are also unlikely to adsorb to solid matrices and are likely to be mobile in the environment.

4.2.2 Volatilisation

The vapour pressure of metazachlor (Gueckel, W (1991) in reference 1) is 9.5 x 10^{-5} Pa at 20 ^{o}C. The calculated Henry's Law Constant (Ohnsorge, U (2000) in reference 1) is 5.865 x 10^{-5} Pa.m^3.mol^{-1} at 20 °C based on measured data. Using predictive software (Battersby, R.V (2000) in reference 1), the estimated Henry's Law Constant is 5.88 x 10^{-6} Pa.m^3.mol^{-1} at 25 ^{o}C.

On this basis metazachlor is considered unlikely to partition to the air. This is supported by the results of volatilisation from soil and plant surface studies which observed low losses (Sarafin, R (1993), Walter, B (1993a), Walter, B (1993b) and Beinhauer, K (1993) in reference 11)

4.2.3 Distribution modelling

Not relevant to this type of dossier.

4.3 Bioaccumulation

4.3.1 Aquatic bioaccumulation

4.3.1.1 Bioaccumulation estimation

Metazachlor has the following measured log K_{ow} values; 2.49 at 21 ^{o}C, pH 7 (OECD guideline 117) (Daum, A (1998) in reference 1); 2.5 at 22 ^{o}C, pH 2.1 and 7 (OECD guideline 117) (Schneider, E (1998) in reference 1). These values are below 3 indicating a low bioaccumulation potential. On this basis a fish aquatic bioaccumulation study has not been conducted.

For example, a BCF_{fish} of 26.6 $l/kg_{wet\ fish}$ can be estimated based on the highest log K_{ow} measurement, following Equation 74 in the Technical Guidance Document (2003) (reference 12). This log K_{ow} value is within the domain of the QSAR (log K_{ow} 2-6).

4.3.1.2 Measured bioaccumulation data

No experimental data are available.

4.3.2 Terrestrial bioaccumulation

Not relevant for this type of dossier.

4.3.3 Summary and discussion of bioaccumulation

Based on the measured log K_{ow} values (<3) and estimated BCF_{fish} (26.6 $l/kg_{wet\ fish}$) metazachlor is considered to have a low bioaccumulation potential.

4.4 Secondary poisoning

Not relevant for this type of dossier.

5 HUMAN HEALTH HAZARD ASSESSMENT

Metazachlor manufactured for use as a pesticide has a minimum purity of 95%. The metazachlor used in the following studies had a purity that was typically ≥ 93.6 %, unless stated otherwise. After careful and detailed review by the UK CA and those authorities responsible for the assessment under Directive 91/414/EEC, these studies have been judged to be adequate for the substance that is being marketed.

5.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

The following summary is derived from the Pesticide Assessment Report made for the review under Directive 91/414/EEC.

All the following toxicokinetic information on metazachlor was acquired from rat studies. Metazachlor was well absorbed (≥ 85 % of the administered dose) following administration of a single oral dose. 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 on one formulation identified an absorption value of 9 %. Absorption via the inhalation route has not been investigated. Distribution after oral absorption was extensive and widespread, with the highest levels of radioactivity generally associated with well-perfused organs, such as the liver and kidney. High levels of radioactivity were also found to be associated with red blood cells. With the exception of the red blood cells, levels of radioactivity in all tissues decreased approximately 24 hours after administration and were low or not detected by 168 hours. The comparative levels of radioactivity in plasma and blood cells and the persistence of blood cell radioactivity are consistent with the covalent binding of metazachlor or a metabolite to blood cells. The data suggest that a metabolite of metazachlor (rather than the parent) may be the species binding to the blood cells. There was also evidence presented that metazachlor covalently binds to proteins in the liver and kidney.
Metazachlor was found to be extensively metabolised, in all studies, by a number of different routes including hydroxylation of the pyrazole ring, oxidation of the methyl moiety or glutathione conjugation with subsequent formation of the mercapturic acid and further oxidation of the sulphur-containing side chain. Metazachlor was rapidly excreted (largely within 24 hours) following a single low dose in all studies. In males, 45-64 % was excreted via the faeces and 28-31 % was excreted via the urine. In females, the situation was somewhat reversed, 39-47 % was excreted via the faeces and 42-57 % was excreted via the urine. A study in bile cannulated animals at a low dose showed biliary excretion of 64 % in males and 52 % in females indicating that the majority of faecal radioactivity is biliary in origin. Following a high dose, excretion was prolonged, but still occurred largely within 48 hours, and the proportions were comparable to those seen with a low dose except that biliary excretion was only 22 % in males and 24 % in females, suggesting saturation. The excretion profile following multiple dosing is broadly comparable to that seen following a single low dose.

5.2 Acute toxicity

5.2.1 Acute toxicity: oral

Table 5.1 Acute toxicity: oral

Species [Reference]	LD_{50} (mg/kg)	Observations and remarks
RAT		

Wistar 5/sex Prakash; 1994a in reference 2	3480 (sexes combined)	OECD 401– vehicle: peanut oil Deaths observed from 2250 mg/kg (lowest dose tested) and generally occurred within 24 hours. Signs of toxicity included lethargy, hypersensitivity to external stimuli and convulsions. Gross necropsy of decedents revealed congestion of the liver and lungs; no treatment related findings were noted in survivors.
Sprague-Dawley 5/sex Merkel; 2002 in reference 2	> 2000 in males 3004 in females	OECD 401 – vehicle: carboxymethylcellulose Deaths observed from 2000 mg/kg (lowest dose tested) and occurred on the day of dosing. Signs of toxicity included hunched posture, hypoactivity, piloerection and reduced faeces. Gross necropsy of decedents revealed discoloration of the lungs and intestinal tract; no treatment related findings were noted in survivors.
Sprague-Dawley 10/sex Leuschner; 1978 a in reference 2	2150 (sexes combined) Purity: not stated	Non-guideline– vehicle: hydroxyethyl cellulose Deaths observed from 1780 mg/kg and occurred on the day of dosing. Signs of toxicity included dyspnoea, apathy, sedation, spasms, abdominal or lateral position, miosis, tremors and convulsions. Gross necropsy of decedents revealed violet urine, dark brown stomach contents and pale kidneys; no treatment-related findings were noted in survivors.
MOUSE		
Swiss albino 5/sex Prakash; 1994b in reference 2	3321 (sexes combined)	OECD 401 – vehicle: peanut oil Deaths observed from 2500 mg/kg (lowest dose tested) and occurred on the day of dosing. Signs of toxicity included tremors and/or convulsions and lethargy. Gross necropsy of decedents revealed congestion of the liver and lungs; no treatment related findings were noted in survivors.
NMRI 10/sex Leuschner 1978b in reference 2	2010 (sexes combined) Purity: not stated	Non-guideline– vehicle: methyl-hydroxyethyl cellulose Deaths observed from 1470 mg/kg and occurred on day of dosing. Signs of toxicity included dyspnoea, apathy, sedation, spasms, abdominal position, mydriaisis, tremors and convulsions. Gross necropsy revealed pale liver; no treatment related findings were observed in survivors.

5.2.2 Acute toxicity: inhalation

Table 5.2 Acute toxicity: Inhalation

Species	LC_{50} (mg/l 4h)	Observations and remarks
RAT		
Sprague-Dawley 10/sex Leuschner, 1978h in reference 2	> 34.5 (both sexes) Purity not stated	Non-guideline- Mean particle size of the dust tested was 0.68 µm; 80 % between 0.1-1.2 µm. No animals died and no signs of toxicity were observed during the study.
Wistar 5/sex Prakash, 1996 in reference 2	> 1.58 (maximum achievable concentration)	OECD 403 - aerosol – Vehicle: DMSO – Mean particle size (0.96 µm; 90 % < 1.42 µm). No deaths occurred. Signs of toxicity included nasal irritation, discharge and lethargy. Gross necropsy did not reveal any treatment-related findings.

5.2.3 Acute toxicity: dermal

Table 5.3 Acute toxicity: dermal

Species	LD_{50}	Observations and remarks

	(mg/kg)	
RAT		
Wistar 5/sex/group Prakash; 1994c in reference 2	> 2000 mg/kg bw	OECD 402 – Vehicle: slurry in distilled water No animals died and no signs of toxicity or local dermal irritation were observed. Gross necropsy did not reveal any treatment relevant findings.
Wistar 5/sex/group Dreher; 2001a in reference 2	> 2000 mg/kg/bw	OECD 402 – Moistened with distilled water No animals died and no signs of toxicity or local dermal irritation were observed. Gross necropsy did not reveal any treatment relevant findings.
Sprague-Dawley 10/sex/group Leuschner, 1978e in reference 2	> 6810 mg/kg/bw Purity not stated	Non-guideline– Substance suspended in water No animals died and no signs of toxicity or local dermal irritation were observed. Gross necropsy did not reveal any treatment relevant findings.

5.2.4 Acute toxicity: other routes

Not relevant

5.2.5 Summary and discussion of acute toxicity

Oral LD_{50} values of > 2000 mg/kg were derived from studies conducted with rats and mice.

Dermal LD_{50} values of > 2000 mg/kg were derived from studies conducted with rats.

For the inhalation route, an LC_{50} > 35 mg/l for 4 hours was derived from one study conducted with rats.

These data indicate that no classification is required under either Directive 67/548/EEC or the CLP Regulation.

Directive 67/548/EEC criteria: no classification proposed

CLP Regulation: no classification proposed

5.2.6 Summary and discussion of specific target organ toxicity – single exposure

There was no evidence of any specific, non lethal target organ toxicity arising from a single exposure to metazachlor (see tables 5.1, 5.2 and 5.3). Clinical signs of toxicity were observed after single exposures to metazachlor but were considered to be non-specific signs of general acute toxicity. In addition, no human data are available that would support classification for this endpoint. No classification as STOT-SE under the CLP Regulation is proposed.

5.3 Irritation

5.3.1 Skin

The skin irritation potential of metazachlor has been investigated in two standard guideline studies (Prakash 1994d and Dreher 2001b both in reference 2). No signs of dermal irritation were observed in any animal at any time point investigated (see section B 6.2.4 of the Pesticide Assessment Report for more information).

5.3.2 Eye

The eye irritation potential of metazachlor has been investigated in three guideline studies (Prakash 1994e, Dreher 2001c and Leuschner 1978g all in reference 2). No effects on the cornea or iris were noted in any study. Effects on the conjunctivae were limited to slight erythema (grade 0.33- 1) observed in all three studies and mild oedema (grade 0.33) observed in one study (see section B.6.2.5 of the Pesticide Assessment Report for more information).

5.3.3 Respiratory tract

Nasal irritation and discharge was observed in one of the two available acute inhalation studies (see section 5.2.2). However, this was a mild and transient effect which was not confirmed at gross necropsy.

5.3.4 Summary and discussion of irritation

No irritation was observed in two well-conducted skin irritation studies and only mild irritation was observed in three guideline eye irritation studies.

Overall, these data do not support classification for skin or eye irritation under either Directive 67/548/EEC or CLP Regulation.

Nasal irritation and discharge was observed in one of the two available acute inhalation studies (see section 5.2.2). However, this was a mild and transient effect which was not confirmed at gross necropsy. In view of this, and taking into account the absence of any significant skin and eye irritation, no classification is proposed for respiratory tract irritation under either Directive 67/548/EEC or CLP Regulation.

Directive 67/548/EEC criteria: no classification proposed

CLP Regulation: no classification proposed

5.4 Corrosivity

There is no evidence from the skin irritation studies that metazachlor is corrosive.

Directive 67/548/EEC criteria: no classification proposed

CLP Regulation: no classification proposed

5.5 Sensitisation

5.5.1 Skin

Table 5.4 Skin sensitisation

Species	Guideline/ purity	Method	Number of animals sensitized/total number of animals	Result
Guinea-pig/ Dunkin Hartley Gelbke & Grundler, 1980 in reference 2	Non-guideline– maximisation study	Induction: Intradermal: 20% ± FCA erythema and necrosis observed Topical: 50% in water + 10% SDS Erythema, oedema and ulceration observed Challenge: 50% in water	16/16 test 0/6 control No positive control	Positive
Guinea-pig/ Dunkin- Hartley Dreher, 2001d in reference 2	OECD 406- Buehler	Induction: 50% in arachis oil 3 applications: no irritation observed Challenge: 50 and 25% in arachis oil	0/20 test 0/10 control Positive control: 100% α-Hexylcinnamaldehyde – 3/20 animals sensitised	Negative
Guinea-pig/ NIH-Hartley Purshottam; 1996 in refence 2	OECD 406 – Buehler	Induction: 0.5g moistened in water 3 applications: no irritation observed Challenge: 0.5 g moistened in water	0/20 test 0/10 control Positive control: Mercaptobenzothiazole (% not given) – 3/5 animals sensitised	Negative
Guinea-pig/ Dunkin Hartley Grundler & Kirsch, 1981 in reference 2	Non-guideline– open Epicutaneous test	Induction: 2, 5, 15 or 40% in water 5 days/wk for 4 wks; grade 1 erythema noted in 1 animal during induction Challenge: 2, 5, 15 or 40% in water on day 3 and 17 post induction	0/8 test 0/8 control No positive control	Negative

The potential of metazachlor to induce skin sensitisation has been investigated in four guinea pig studies.

In a maximisation study, a positive response was observed in all animals. None of the negative control animals displayed any adverse skin reactions. The results of two Buehler studies were negative. However, in one study, only a weak positive response was observed with the positive control substance, 100% α-Hexylcinnamaldehyde, undermining the significance of this negative result (Dreher 2001d in reference 2).

In an open epicutaneous assay, no reactions occurred in the test group animals. It was not reported if positive control animals were included, or if they were, if they responded appropriately.

5.5.2 Respiratory system

No data

5.5.3 Summary and discussion of sensitisation

Metazachlor was positive in a well-conducted Guinea-pig maximisation study, but negative in two Buehler and an open epicutaneous study. The maximisation test is generally considered to be the more rigorous and sensitive of these types of test, on account of the use of an adjuvant and occlusive dressing; therefore, the findings from this test take precedence.
Overall, given the clearly positive findings in the maximisation test (i.e. clear responses in greater than 30 % of animals), classification with Xi; R 43 under Directive 67/548/EEC and for skin sensitisation category 1 (H317) under the CLP regulation are proposed.

There is no available information on the potential of the test substance to induce respiratory sensitisation.

Directive 67/548/EEC criteria: propose R43

CLP Regulation: propose H317

5.6 Repeated dose toxicity

5.6.1 Repeated dose toxicity: oral

5.6.1.1 Rat

There are 6 studies available: three 28-day studies and three 90-day studies

(i) 28 day studies

Table 5.5 Repeat dose: 28-day studies in rat

Dose schedule [reference]	Dose levels	Observations and remarks (effects of major toxicological significance)
Daily in the diet Wistar 5/sex/group OECD 407 Suresh, 1995 in reference 2	0, 100, 1250 or 15000 ppm Corresponds to 8, 109 and 1356 mg/kg/day in males 10, 131 and 1483 mg/kg/day in females	15000 ppm: 19 % ↓ bodyweight (male), 12/15 % ↓ food consumption (male/female), 20/30 % ↑ absolute liver weight (male/female), 60/45 % ↑ relative liver weight (male/female), 33 % ↑ relative kidney weight (male), 56 % ↓ neutrophils (male), 15 % ↑ lymphocytes (male) 1250 ppm: 13 % ↑ relative kidney weight (male), 45 % ↓ neutrophils (male), 13 % ↑ lymphocytes (male) 100 ppm : Noadverse effects NOAEL of 109 (males) and 131(females) mg/kg/day
Daily in the diet Sprague- Dawley 25/sex/group Range finding:	0, 3600, 10800 or 32400 ppm Corresponds to 0, 289, 862 or 3241 mg/kg/day in	32400 ppm: 50/40 % ↓ bodyweight (male/female), 70/89 % ↑ relative liver weight (male/female), 32/24 % ↑ relative kidney weight (male/female) 1080 ppm:16 % ↓ bodyweight (male), 29/17 % ↑ relative liver weight (male/female), 22 %↑ relative kidney weight (male)

Non-guideline Leuschner et al, 1978 in reference 2	males 0, 286, 857 or 3030 mg/kg/day in females Purity: 91%	3600 ppm: 12 %↑ relative liver weight (female), 12 %↑ relative kidney weight (male) NOAEL of 289 mf/kg/day for males and a LOAEL of 286 mg/kg/day for females
Daily in the diet Wistar 5/sex/group Range finding Non-guideline Malleshappa, 2001 in reference 2	0, 600, 3000 or 15000 ppm. Corresponds to 0, 54, 281 and 1357 mg/kg/day in males 0, 56, 282 and 1472 mg/kg/day in females.	15000 ppm: 23/14 % ↓ bodyweight (male/female), 24/16 % ↓ Food consumption (male/female), 20/46 % ↑ absolute liver weight (male/female), 62/70 % ↑ relative liver weight (male/female), 21/22 % ↑ relative kidney weight (male/female) 3000 ppm: 9 % ↓ Food consumption (male), 12 % ↓ bodyweight (male), 18/14 % ↑ relative liver weight (males), 11 % ↑ in relative kidney weight (male) 600 ppm: no adverse effects NOAEL = 54 (males) and 56 (females) mg/kg/day

NB: The values for NOAEL and LOAEL are provided for information only: they have already been agreed at a PRAPER expert meeting.

No adverse effects were observed below the harmful (Xn) sub-acute cut-off value of 150 mg/kg/day in two 28-day studies. In a third study, the only effects observed below the cut-off value were an increase in kidney weight and some changes in blood parameters in males at 109 mg/kg/day. These effects are not considered to represent a clear functional disturbance or morphological change of toxicological significance and, hence, serious damage to health. At dose levels above the cut-off value (up to 3241 mg/kg/day) effects included reductions in bodyweight and food consumption and increases in liver and kidney weight. These organ weight changes were not accompanied by any histopathology.

(ii) 90 day studies

Table 5.6 Repeat dose study: 90-day studies in rat

Dose schedule [reference]	Dose levels	Observations and remarks (effects of major toxicological significance)
Daily in the diet Wistar 10/sex/group OECD 408 Suresh, 1996 in reference 2	0, 300, 2000 or 15000 ppm Corresponds to 21, 147 and 1186 mg/kg/day in males 0, 30, 299 and 1559 mg/kg/day in females	15000ppm: 16/10 % ↓ bodyweight (male/female), occasional ↓ food consumption (male), 26/40 % ↑ absolute liver weight (male/female), 52/55 % ↑ relative liver weight (male/female), 23/16 % ↑ relative kidney weight (male/female), 17/11% ↓ in mean corpuscular haemoglobin (male/female), 15 % ↓ in mean corpuscular haemoglobin concentration (male), 44/33 % ↑ bilirubin (male/female), hepatic centrilobular fatty change (2/10 males v 0 in control). 2000 ppm: 19 % ↑ absolute liver weight (female), 7/22 % ↑ relative liver weight (male/female), 9/14 % ↑ relative kidney weight (male/female), 14 % ↓ in mean corpuscular haemoglobin (female), 44 % ↑ bilirubin (male) 300 ppm: no adverse effects NOAEL= 21 (males) and 30 (females) mg/kg/day
Daily in the diet Wistar 10/sex/group OECD 408 Malleshappa, 2002 in reference	0, 250, 1250 and 7500 ppm. Corresponds to 0, 17, 84 and 526 mg/kg/day in males 0, 20, 98 and 582 mg/kg/day in	7500 ppm: 13 % ↓ bodyweight (male), 36/23 %↓ food consumption in week 1 (male/female), 28/19 % ↓ bodyweight gain (male/female), 31% ↑ absolute liver weight (female), 25/39 % ↑ relative liver weight (male/female), 17/12 % ↑ relative kidney weight (male/female), hepatocyte hypertrophy (6/10 males and 2/10 females v 0 in control), increases splenic heamosiderosis (2/10 females v 0 in control) 1250 ppm: 10/12 % ↑ relative liver weight (male/female)

2	females	 250 ppm: no adverse effects NOAEL = 17(females) and 20 (males) mg/kg/day
Daily in the diet Sprague-Dawley 25/sex/group Non-guideline Leuschner, 1979a in reference 2	0, 1200, 3600 and 10800 ppm. Corresponds to 0, 110, 330 and 989 mg/kg/day in males and females 91 % purity	10800 ppm: 15/13 % ↓ bodyweight (male/female), ↓ food consumption, 28/27 % ↑ absolute liver weight (male/female), 48/49 % ↑ relative liver weight (male/female), 13/16 % ↑ absolute kidney weight (male/female) 55/72 % ↑ aspartate transaminase (male/female), diffuse fatty hepatocyte degeneration (8/25 males v 0 in control) and renal ectasis (7/25 males v 0 in control) 3600 ppm: 10 % ↓ bodyweight (male/female), 10 % ↑ absolute liver weight (male), 13 % ↑ relative liver weight (male) 1200 ppm: no adverse effects NOAEL of 110 (males) and 330 (females) mg/kg/day

NB: The values for NOAEL and LOAEL are provided for information only: they have already been agreed at a PRAPER expert meeting

No adverse effects were observed below the harmful (Xn) sub-chronic classification cut-off value of 50 mg/kg/day in the three available studies in the rat. At dose levels above the cut-off value (up to 1559 mg/kg/day) effects included reductions in bodyweight and food consumption, increases in liver and kidney weights, haematological (↓ mean corpuscular haemoglobin) and clinical chemistry (↑ bilirubin, ↑ aspartate transaminase) changes, and histopathological findings of the liver (hypertrophy and fatty degeneration), spleen (haemosiderosis) and kidney (renal ectasis).

Summary of oral data in the rat

In the available sub-acute and sub-chronic studies in the rat, no serious adverse effects were observed below the harmful (Xn) cut-off values for classification. At dose levels above the cut-off values the liver, kidney, and spleen (with associated haematological changes) were the target organs of toxicity of metazachlor. In the three available chronic studies in the rat (see section 5.8.11), serious adverse effects (histopathological findings in the liver, kidney and spleen) were only seen at relatively high dose levels (200-300 mg/kg/day) of no relevance for classification.

5.6.1.2 Mouse

There is one 28-day study available for the mouse.

(i) 28-day study

Table 5.7 Repeat dose studies: 28-day studies in mice

Dose schedule [reference]	Dose levels	Observations and remarks (effects of major toxicological significance)
Daily in the diet CD-1 8/sex/group Non-guideline (range-finding study) Hunter, 1980 in reference 2	0, 2500, 5000 and 10 000 ppm Corresponds to 0, 348, 805 and 1569 mg/kg/day in males 0, 379, 891 and 843 mg/kg/day in females	10 000 ppm: 11 % ↓ bodyweight (male), 24/15 % ↑ absolute liver weight (male/female), 40/24 % relative liver weight (male/female), 5000 ppm: 12 % ↑ absolute liver weight (female), 20/25 % ↑ relative liver weight (male/female), 2500 ppm: 11 % ↑ absolute liver weight (female) NOAEL – 348 (males) and 379 (females) mg/kg/day

NB: The values for NOAEL and LOAEL are provided for information only: they have already been agreed at a PRAPER expert meeting

In the only study available in the mouse (28-day study) effects were limited to a significant increase in absolute and relative liver weight. However, these changes were only statistically significant in males administered a high dose (1569 mg/kg/day).

5.6.1.3 Dog

There are 8 studies available for the dog: three of 28-day duration, two of 90-day duration, two of 6 month and one of 1-year duration.

(i) 28-day studies

Table 5.8 Repeat dose studies: 28-days in dogs

Dose schedule [reference]	Dose levels	Observations and remarks (effects of major toxicological significance)
Daily gavage Vehicle: water Beagle 4/sex/group Non-guideline Leuschner, 1979c in reference 2	0, 30, 90 and 270 mg/kg/day 91% purity	270 mg/kg/day: weight loss in males (-0.350 kg v 0.025 kg in controls) and females (-0.975 kg v -0.1 kg in controls). 20/17 % ↑ in absolute liver weight (male/female), 40/46 % ↑ in relative liver weight (male/female), 59/81 % ↑ alkaline phosphatase (male/female) Slight thymic atrophy was observed in all dose groups although a clear dose response was not observed- therefore not considered treatment related 90 mg/kg/day and 30 mg/kg/day: No adverse effects NOAEL – 90 mg/kg/day in both sexes
Daily in the diet Beagle 1/sex/group Non-guideline Prakash, 2000 in reference 2	0, 100, 1000, 5000 or 10 000 ppm Estimated to be 2.5, 25, 125, 250 mg/kg./day	10 000 ppm: weight loss in males (-0.2 kg v 0.7 kg in control) and females (-0.7 kg v 0.4 kg in control), ↓ Food consumption (male/female) 5000 ppm: weight loss in females (- 0.4 kg v 0.4 kg in control), ↓ Food consumption (female) 1000 ppm and 100 ppm: no adverse effects NOAEL – 25 mg/kg/day in both sexes
Daily in the diet Beagle 2/sex/group Non-guideline Leuschner 2002 in reference 2	300 or 450 mg/kg/day	450 mg/kg/day: ↓ Food consumption (male/female), -2/-3 kg weight loss (male/female) 300 mg/kg/day: ↓ Food consumption (male/female), 3/2 kg weight loss (male/female) A number of clinical chemistry changes were observed in each group. However, in the absence of a control it is difficult to determine the significance or extent of these effects. Due to the absence of a control group, a NOAEL could not be derived for this study

NB: The values for NOAEL and LOAEL are provided for information only: they have already been agreed at a PRAPER expert meeting

In the available 28-day studies in the dog, adverse effects started to emerge from a dose of 250 mg/kg/day. Effects consisted of reductions in food consumption, body weight loss and increases in liver weight with associated increases in the level of serum alkaline phosphatase. In one study, reduction in food consumption and bodyweight loss was also observed at a dose level of 125

mg/kg/day (females only). However, the reliability of these findings is limited by the small group size (1 animal/sex/group).

(ii) 90-day studies

Table 5.9 Repeat dose study: 90-days in dogs

Dose schedule [reference]	Dose levels	Observations and remarks (effects of major toxicological significance)
Daily via gavage Beagle 4/sex/group Non-guideline Leuschner, 1976b in reference 2	0, 30 , 90, 270 mg/kg/day Purity: 91 %	270 mg/kg/day: bodyweight loss in males (-0.2 kg v 0.7 kg in control) and females (-0.08 kg v 0.96 kg in control), 9/10 % ↑ absolute liver weight (male/female), 16/17 % ↑ relative liver weight, 86/83 % ↑ alkaline phosphatase (male/female) 90 mg/kg/day and 20 mg/kg/day: no adverse effects observed A NOAEL of 90 mg/kg/day was derived
Daily in diet Beagle 4/sex/group OECD 409 Leuschner, 2003 in reference 2	Intake measured as 0, 49, 142 and 356 mg/kg/day in males 0, 48, 129, 342 mg/kg/day in females	356/342 mg/kg/day: One female died on day 50, 58/47 % ↓ food consumption (male/female), weight loss in males (1.88 kg v 1.7 kg in controls) and females (-1.48 kg v 0.6 kg in controls), 21/70 % ↑ absolute liver weight (male/female), 58/90 % ↑ relative liver weight (male/female), 27/59 % ↑ absolute kidney weight (male/female), 66/77 %, ↑ relative kidney weight (male/female), 50 % ↑ relative thyroid weight (male), 21 % ↓ red blood cells (male), 10 % ↓ in mean corpuscular volume (male), 235 % ↑ alkaline phosphatase (male) 62 % ↓ alanine transaminase (female), 24 % ↓ creatine (female), 38 % ↓ bilirubin (male), ↑severity of swollen hepatocytes compared to the control. 142/129 mg/kg/day: 45/43 % ↓ food consumption (male/female), 15/25 % ↑ absolute liver weight (male/female), 24/16 % ↑ absolute kidney weight (male/female), 35/19 % ↑ relative kidney weight (male/female) 49/48 mg/kg/day: 28 % ↓ food consumption (female)*, 24 % ↑ absolute liver weight (female), 22/10 % ↑ absolute kidney weight (male/female), 21 % ↑ relative kidney weight (male)** A NOAEL of 50 mg/kg/day was derived for both sexes

** Reduction in food consumption at ~50 mg/kg/day was not considered adverse due to the magnitude and no corresponding decrease in bodyweight.*
*** The effects on organ weights at ~50 m/kg/day were not considered adverse due to the magnitude and lack of statistical significance*
NB: The values for NOAEL and LOAEL are provided for information only: they have already been agreed at a PRAPER expert meeting

In the available 90-day studies in the dog, adverse effects started to occur from a dose of 129 mg/kg/day. At this dose level, effects consisted of reductions in food consumption and increase in liver and kidney weights. At higher dose levels (around 270-350 mg/kg/day) bodyweight loss, clinical chemistry changes (↑ alkaline phosphatase, ↓ creatine, ↓ bilirubin and ↓ alanine transaminase), haematological changes (↓ red blood cells and ↓ mean corpuscular volume), increased thyroid weight and liver hypertrophy were also observed. In one study, one (out of four) female died at 342 mg/kg/day.

(iii) 6-month and 12 month studies

Table 5.10 Repeat dose studies: 6-month and 12 month in dogs

Dose schedule [reference]	Dose levels	Observations and remarks (effects of major toxicological significance)

6 month study Daily in the diet Beagle dogs 6/sex/group No guideline (acceptable) Kirsch P et al, 1981a in reference 2	0, 1000, 3000 or 9000 ppm Estimated to be 25, 75 and 225 mg/kg/day in both sexes	9000 ppm: one female died, vomiting was observed, 14/41 % ↓ in food consumption, 32/38 % ↓ bodyweight (male/female), 48/29 % ↑ in relative liver weight (male/female), 73/100 % ↑ in relative kidney weight (male/female), 23/15 % ↓ red blood cells (male/female), 19/14 % ↓ haemoglobin (male/female), 19/13 % ↓ haemocrit (male/female), 171/ 61 % ↑ reticulocytes (male/female), 30 % ↑ bilirubin (male), 17/21 % ↓ Creatine (male/female), 51/63 % ↓ triglycerides (male/female), 217 % ↑ alkaline phosphatase (female), pale kidney (6 males), bile duct degeneration (5 males/4 females), kupffer cell siderosis (4 male/5female v 0 in control), liver siderosis (1 female v 0 in control), Kidney degeneration (5 male, 2 female v 0 in control), Bone marrow atrophy (1 male/1 female v 0 in control), spleen siderosis (↑ incidence and severity in both sexes compared to control) 3000 ppm: 23 % ↑ relative liver weight (male), 33/23 % ↑ relative kidney weight (male/female), 10 % ↓ red blood cells (male), 8 % ↓ haemoglobin (male), 11 % ↓ haemocrit (male), pale kidney (3 males v 0 in control), bile duct degeneration (4 male/1 female v 0 in control), kupffer cell siderosis (2 male/4 female v 0 in control), liver siderosis (1 female v 0 in control), kidney degeneration (6 male, 2 female v 0 in controls), spleen siderosis (↑ incidence and severity in both sexes compared to the controls) 1000 ppm: 18 % ↑ relative kidney weight (male), Bile duct degeneration (2 male v 0 in controls), kidney degeneration (3 male v 0 in controls), spleen siderosis (↑ incidence and severity in both sexes compared to the controls) A LOAEL of 25 mg/kg/day was derived for both sexes (lowest dose tested)
6 month study Daily in the diet Beagle 6/sex/group Non-guideline (acceptable) Kirsch et al, 1981b	0 or 200 ppm 0, 6.3 mg/kg/day in males 0, 6.4 mg/kg/day in females	200 ppm: slight weight loss observed in one male, but group bodyweight means were unaffected by treatment. No treatment related effects were observed in haematology, clinical chemistry, urinalysis, organ weights or at necropsy. A NOAEL of 6.3 (males) and 6.4 mg/kg/day (females) was derived
12 month study Daily in the diet Beagle 5/sex/group OECD 452 Wiemann et al, 2002 in reference 2	0, 200, 1000 or 5000 ppm Corresponds to 0, 6, 29 and 148 mg/kg/day in males 0, 6, 31 and 159 mg/kg/day in females	5000 ppm: ↓ in food consumption (consistently in males up to day 240 weight/ intermittently in females) , weight loss in males (-0.3 kg v 0.2 kg in controls) and females (-0.4 kg v 0.1 kg in controls), 24/38 % ↑ absolute liver weight (male/ female), 26/40 % ↑ relative liver weight (male/female), 45/54 % ↑ absolute kidney weight (male/female), 46/59 % ↑ relative kidney weight (male/female), 13 % ↓ red blood cells at 3 months (female), 18% ↓ red blood cells at 6 months (female), 7-14 % ↑ mean corpuscular volume (female), 58-180 % ↑ in alkaline phosphatase (female), 25 % ↑ in urea (male), 22/16 % ↑ creatine (male/female), 68 % ↑ triglycerides (male). Prominent acini (2 male/1 female v 0 in control), single cell necrosis in liver (3 male and 2 female v 0 in control), cirrhosis of liver (2 male/ 1 female v 0 in control), bile duct proliferation (4 male/ 1 female v 0 in control), enlarged spleen (1 male/ 2 female v 0 in control), spleen haemosiderosis (1 male/ 2 female v 0 in control), spleen haemotopoiesis (1 male/ 2 female v 0 in control), increased bone marrow myelopoiesis (2 female v 0 in control) 1000 ppm: 18 % ↑ relative kidney weight (female), bile duct proliferation (1 male v 0 in control), liver cell infiltration (1 male v 0 in control) 200 ppm: No adverse effects A NOAEL of 29 (males) and 31 (females) mg/kg/day was derived (lowest dose tested)

NB: The values for NOAEL and LOAEL are provided for information only: they have already been agreed at a PRAPER expert meeting

In the available non-guideline 6 month studies in the dog, adverse effects started to occur from a dose of 25 mg/kg/day. At this dose level, effects consisted of a small increase in kidney weight and low incidences of histopathological findings in the liver (fatty degeneration of bile duct), kidney (degeneration) and liver (kupffer cell siderosis). At the next dose level (75 mg/kg/day), increases in liver and kidney weights, haematological (↓ haemoglobin, ↓ haemocrit) and clinical chemistry changes (↑ creatine) were also observed. At the highest dose (225 mg/kg/day) there were also reductions in food consumption, body weight loss, one mortality, clinical signs of toxicity and additional changes in haematology and clinical chemistry.

In a subsequent guideline 12-month dog study, adverse effects started to occur from a dose of 29 mg/kg/day. At this dose level, the only effects consisted of a small increase in kidney weight and low incidences of minor histopathological findings of the liver (bile duct proliferation and cell infiltration). At the next dose level (150 mg/kg/day) there were also reductions in food consumption, bodyweight loss, increases in liver and kidney weights, haematological (↓ red blood cells, ↑ mean corpuscular volume) and clinical chemistry changes (↑ alkaline phosphatase, ↑ urea, ↑ creatine, ↑ triglycerides), more severe histopathological findings of the liver (single cell necrosis and cirrhosis) and histopathological changes in the spleen (siderosis and extreme medullary haematopoiesis) and femoral bone (myelopoiesis).

Overall, therefore, in the dog there were serious adverse effects (histopathological changes of the liver, kidney and spleen) of metazachlor in a non-guideline 6-month study at dose levels (25 mg/kg/day) below the harmful (Xn) classification cut-off value of 50 mg/kg/day used for rat data obtained from 3-month studies. However, as these effects were not replicated in a longer duration guideline study (12- months) at similar dose levels, but were only seen at a much higher dose (150 mg/kg/day), it can be concluded that serious health effects are seen in the dog only at relatively high dose levels of no relevance for classification.

Summary of the oral data in the dog

Overall, in the available studies in the dog, serious adverse effects were only seen at relatively high dose levels (from 250 mg/kg/day in the 28-day studies, from 270 mg/kg/day in the 90-day studies and from 150 mg/kg/day in the 12 month study). These effects indicated that the liver, kidney and spleen (with associated anaemia) are the target organs of toxicity of metazachlor in the dog.

5.6.2 Summary and discussion of oral repeated dose toxicity

The oral repeat dose toxicity of metazachlor has been investigated in three species, the rat, mouse and dog.

The rat data show that there are no serious adverse effects of metazachlor below the harmful (Xn) sub-acute and sub-chronic classification cut-off values and that effects in three different target organs (liver, kidney and spleen) occur only at relatively high dose levels. The mouse data confirm that the liver is a target organ of toxicity of metazachlor at high doses (1600 mg/kg/day in a 28-day study). The dog data also shows that the liver, kidney and spleen are the target organs of toxicity of metazachlor, but that serious adverse effects in these organs only occur at relatively high dose levels of no relevance for classification.

Overall, therefore, the available information indicates that classification for oral repeat dose toxicity is not warranted.

Under the CLP Regulation, the harmful (Xn) classification cut-off values (guidance values) are higher: 100 mg/kg/day for a 90-day study and 300 mg/kg/day for a 28-day study in rats. However, as there were no serious effects below either of these guidance values in all three species investigated, classification for STOT- RE under the CLP Regulation is not warranted.

Directive 67/548/EEC criteria: no classification proposed

CLP Regulation: no classification proposed

5.6.3 Repeated dose toxicity: inhalation

No data available.

Directive 67/548/EEC criteria: no classification proposed

CLP Regulation: no classification proposed

5.6.4 Repeated dose toxicity: dermal

5.6.4.1 Rat

There is one 28-day dermal toxicity study in rats

Table 5.11 Repeat dose toxicity; 28-day in rat

Dose schedule [reference]	Dose levels	Observations and remarks (effects of major toxicological significance)
28-day study 6 h semi-occlusive for 5d/wk Wistar OECD 410 Mellert, 2001a in reference 2	0, 60, 300 and 1000 mg/kg/day	No treatment related changes were observed in bodyweight, clinical chemistry, haematology, organ weight or at necropsy. A NOAEL of 1000 mg/kg/day was derived for both sexes

NB: The values for NOAEL and LOAEL are provided for information only: they have already been agreed at a PRAPER expert meeting

5.6.5 Summary and discussion of dermal repeated dose toxicity

No effects were observed following dermal exposure (28-day study) up to 1000 mg/kg/day (highest dose tested). Therefore, classification is not warranted

Directive 67/548/EEC criteria: no classification proposed

CLP Regulation: no classification proposed

5.6.6 Other relevant information

No data

5.7 Mutagenicity

The mutagenicity of metazachlor has been adequately investigated *in vitro* and *in vivo*.

5.7.1 *In vitro* data

Table 5.12 *In vitro* genotoxicity results

Method Reference	Strain	Dose range Cytotoxicity	Result	
			+S9	-S9
Ames OECD 471 (1981) Shivaram, 1994 in reference 2	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and TA 1538	Five concentrations between 50 – 4500 µg/plate Cytotoxicity (slight thinning of background lawn) observed at 4500 µg/plate	Negative	Negative
Ames OECD 471 Leuschner, 2001a in reference 2	*Salmonella typhimurium* TA98, TA100, TA102, TA1535 and TA 1537	Five concentrations between 100-5000 µg/plate	Negative	Negative
Ames OECD 471 (1997) Zeller & Hendlehardt, 1979 in reference 2	*Salmonella typhimurium*-TA98, TA100, TA1535, TA1537 *Escheririca Coli*-WP2*uvr*A	Seven concentrations between 5- 5000 µg/plate Confirmation Assay: five concentrations 50-5000 µg/plate	Negative	Negative
Ames OECD 471 (1997) Engelhardt & Leibold, 2005 in reference 14	*Salmonella typhimurium*-TA98, TA100, TA1535, TA1537 *Escheririca Coli*-WP2*uvr*A	Five concentrations between 20 – 5000 ug/plate Results confirmed in a second independent assay Appropriate positive controls confirmed the sensitivity of the assay	Negative	Negative

<table>
<tr><td>Ames

81.5 % purity

Schulz & Landsiedel, 2006 in reference 14</td><td>Salmonella typhimurium- TA98, TA100, TA1535, TA1537

Escheririca Coli- WP2uvrA</td><td>Five concentrations between 26 – 5000 ug/plate

Results confirmed in a second independent assay

Appropriate positive controls confirmed the sensitivity of the assay</td><td>Negative</td><td>Negative</td></tr>
<tr><td>Mammalian cell gene mutation

EEC 79/831

Tippins, 1984 in reference 2</td><td>Chinese hamster V79 cells</td><td>5, 9, 18, 35 & 70 µg/ml (-S9) and 6, 13, 25, 50 & 100 µg/ml (+S9)

Cytotoxicity not up to the degree required by guideline:
33 % relative survival was observed at 70 ug/ml (-S9) and 40 % relative survival was observed at 100 ug/ml (+S9)</td><td>Negative</td><td>Negative</td></tr>
<tr><td>Mammalian cell gene mutation

OECD 476

Deviation - single cultures

Leuschner, 2001b in reference 2</td><td>L5178Y mouse lymphoma cells</td><td>94, 188, 375, 750 and 1500 µg/ml

Precipitate was observed at 1500 µg/ml.

Cytotoxic level required by the guideline only reached – S9.
Relative survival was < 20 % at 750 µg/ml (-S9) and ≤ 30 % at 1500 µg/ml (+S9)</td><td>Negative</td><td>Equivocal

A slight increase in mutant frequency was observed in the initial and repeat assay. This increase was limited to the highest analysable concentration which was also cytotoxic to the cells. No change in the ratio of small to large colonies was observed in any concentration group.

<table><tr><th rowspan="3">Dose (µg/ml)</th><th colspan="4">-S9</th></tr><tr><th colspan="2">1st Assay</th><th colspan="2">2nd Assay</th></tr><tr><th>MF</th><th>RS</th><th>MF</th><th>RS</th></tr><tr><td>0</td><td>13.3</td><td>100</td><td>14.9</td><td>100</td></tr><tr><td>93.75</td><td>13.3</td><td>66</td><td>21.9</td><td>77</td></tr><tr><td>187.5</td><td>16.3</td><td>35</td><td>14.2</td><td>84</td></tr><tr><td>375</td><td>14.3</td><td>37</td><td>15.7</td><td>27</td></tr><tr><td>750</td><td>25.4</td><td>31</td><td>30.4</td><td>12</td></tr><tr><td>1500</td><td></td><td>13</td><td></td><td>6</td></tr><tr><td>MMS</td><td>397</td><td>21</td><td>445</td><td>17</td></tr></table>
MF- mutation frequency
RS – relative survival</td></tr>
<tr><td>Chromosome aberration

EEC 79/831

Mosesso 1984 in reference 2</td><td>Chinese hamster ovary (CHO) cells</td><td>3, 10 and 30 µg/ml (-S9) and 40, 126 and 400 µg/ml (+S9)

Cytotoxicity not up to the degree required by guideline.
Mitotic index reduced ~35-50% at top concentration</td><td>Negative</td><td>Negative</td></tr>
</table>

The genotoxicity of metazachlor has been tested *in vitro* in five Ames tests, two mammalian cell gene mutation assays and a chromosomal aberration study. Positive controls were included in all assays and showed the expected responses. No evidence of mutagenicity was found in the five Ames tests reported. In one mammalian cell gene mutation study, in the absence of S9, a slight increase in mutation frequency was observed at the highest analysable concentration (750 μg/ml) in two independent experiments (Leuschner, 2001b). These increases were reported to be within the normal control range (not provided) and were only observed at cytotoxic concentrations; as such, the result of this study is considered equivocal. The results of the other mammalian cell gene mutation assay and an *in vitro* chromosome aberration study were negative.

5.7.2 *In vivo* data

(i) Somatic cell

Table 5.13 *In vivo* somatic cell genotoxicity results

<table>
<tr><th>Method</th><th>Strain</th><th>Concentrations test</th><th>Result</th></tr>
<tr><td>Micronucleus study (Bone marrow)

Single dose

OECD 474

Engelhardt & Hoffman, 2001a in reference 2</td><td>Mouse
NMRI
Male
5/dose</td><td>0, 19, 38 or 75 mg/kg
intraperitoneal</td><td>Negative

Signs of toxicity included squatting posture at 19 mg/kg and ptosis and ‘poor general state of health’ at 38 and 75 mg/kg/day

No effect on the P/N ration was observed.</td></tr>
<tr><td>Micronucleus Study (bone marrow)

Single dose

OECD 474 (1983)

Ponnana, 1996a in reference 2</td><td>Mouse
Swiss
Male + female
5/sex/group</td><td>0, 300, 800 or 2300 mg/kg oral gavage (single dose)</td><td>Negative

A dose-related decrease in the PCE:NCE ratio suggesting exposure of the bone marrow

Deviations from current guideline– only 1000 PCEs/animal scored and bone marrow was only sampled at 24 hours.</td></tr>
<tr><td>Chromosomal aberration test (bone marrow)

Single dose

OECD 475

Ponnana, 1996b in reference 2</td><td>Mouse
Swiss
Male + female
5/sex/group</td><td>0, 300, 800 or 2300 mg/kg oral gavage (single dose)</td><td>Equivocal

Significant toxicity was seen at 2300 mg/kg; one male and one female died and lesions were seen in the liver and lungs. Similar lung and liver lesions were seen at 800 mg/kg.

A clear decrease in mitotic index was seen at 2300 mg/kg/day.

<table>
<tr><th rowspan="2">Dose level (mg/kg bw)</th><th colspan="2">Males</th><th colspan="2">Females</th><th colspan="2">M + F</th></tr>
<tr><th>MI</th><th>CA
Minus gaps</th><th>MI</th><th>CA
Minus gaps</th><th>MI</th><th>CA
Minus gaps</th></tr>
<tr><td>0</td><td>17.88</td><td>0</td><td>16.96</td><td>0</td><td>17.42</td><td>0</td></tr>
<tr><td>300</td><td>14.98</td><td>3</td><td>12.51</td><td>2</td><td>13.75</td><td>5</td></tr>
<tr><td>800</td><td>10.06</td><td>3</td><td>9.65</td><td>0</td><td>9.86</td><td>3</td></tr>
<tr><td>2300</td><td>7.49</td><td>1</td><td>7.27</td><td>4</td><td>7.38</td><td>5</td></tr>
<tr><td>+control</td><td>3.93</td><td>63</td><td>3.34</td><td>31</td><td>3.63</td><td>94</td></tr>
</table>
CA: Percent of metaphases with chromosome aberrations (minus gaps</td></tr>
</table>

			per 250 scored) All CA values were within the historical control range for this laboratory (2-12 in males, 0-10 in females and 2-22 in both sexes combined over the period 1993-1995). The aberrations observed were breaks. No complex rearrangements were observed. Deviations from current guideline: mitotic index was only calculated using 100 (rather than 1000) cells and 50 (rather than 100) cells were assessed for chromosomal aberrations and bone marrow was harvested at a single time point
Sister Chromatid Exchange Single dose Non-guideline Gelbke & Engelhardt, 1981b in reference 2	Hamster Chinese Male + female 5/sex/group	0, 3160, 4640 mg/kg oral gavage (single dose)	Negative Animals administered metazachlor exhibited signs of toxicity (dyspnoea and excitation) from 15 minutes to 4 hours following administration.

(ii) Germ cell

Table 5.14 *In vivo* germ cell genotoxicity studies

Method	Strain	Concentrations test	Result
Dominant Lethal Assay Repeat dosing on 5 consecutive days Non-guideline Cozens et al, 1980b in reference 2	Mice CD-1 Male 6/dose	0, 67, 200 or 600 mg/kg, oral gavage	Negative Signs of toxicity were limited to a single incidence of tremor in one top dose male following the first dose. Deviation: no positive control

Four studies have evaluated the potential of metazachlor to induce cytogenetic damage in the bone marrow of mice. No evidence of micronucleus formation was found in two adequate micronucleus studies following either intraperitoneal (Engelhardt & Hoffman, 2001a in reference 2) or oral administration (Ponnana, 1996a in reference 2). In both these studies the test substance was judged to have reached the target organ. In a chromosome aberration study in mice (oral dosing) there were occasional small increases in the frequency of chromosomal aberrations (plus and minus gaps) in some dose groups. This was most evident when male and female data were combined. However, there seemed to be a lack of consistency across males and females and no clear dose response was observed. Also, the increased frequencies were well within the historical control range for this laboratory. In view of this and taking into account that the aberrations observed were breaks, these findings are not considered to provide clear evidence of an *in vivo* mutagenic response. The results of an adequate sister chromatid exchange study were also negative and no evidence of mutagenicity was observed in a germ cell dominant lethal assay.

Overall, the results of these studies provide reassurance that metazachlor has no *in vivo* mutagenic potential.

5.7.3 Human data

No data

5.7.4 Other relevant information

No data

5.7.5 Summary and discussion of mutagenicity

Data indicate that metazachlor is not mutagenic *in vitro* or *in vivo* and does not meet the criteria for classification as a mutagen.

Directive 67/548/EEC criteria: no classification proposed

CLP Regulation: no classification proposed

5.8 Carcinogenicity

There are three carcinogenicity studies available in the rat and two studies available in the mouse.

In addition, following the completion of the Pesticide Assessment Report, industry submitted new information on this endpoint, namely mechanistic studies (see section 5.8.5) and a re-evaluation of the neoplastic findings.

With regards the re-evaluation, the histopathological sections, from all animals, of the organs in which increases in tumour findings had been identified by the study pathologist were re-examined internally by two BASF pathologists (March 2008) and an external reviewer (mouse bladder tumours only: 13 December 2007). The BASF pathologists applied the most up-to-date diagnostic/classification criteria of the WHO nomenclature available at the time (mice: Mohr U, in 2001 in reference 3 in Appendix 1 and rats: Mohr U, in 1997 in reference 1 in Appendix 1) and identified deviating findings from those of the original study results. To resolve these discrepancies a Pathology Working Group (PWG), sponsored by industry, was organised to review the slides from the animals in which critical (neoplastic as well as hyperplastic) findings had been identified by the study pathologist or the BASF pathologists and come to a consensus diagnosis and a final decision. This PWG group consisted of five fully qualified pathologists and one chairman from both independent contract research institutes and academia. The two BASF pathologists involved in the re-evaluation were present during the PWG review as non-voting observers. The PWG examined coded slides without prior knowledge of treatment group. As already stated above, not all slides were re-examined, but only those slides with previous diagnoses and those the Chairman chose for re-examination. The re-examination by the PWG took place in July 2008. The PWG pathologists also used the most up-to-date diagnostic criteria of the WHO nomenclature (mice: Mohr U, in 2001 in reference 3 in Appendix 1 and rats: Mohr U, in 1997 in reference 1 in Appendix 1). These criteria reflect recent advances in the scientific knowledge on the development of tumours, gain in experience in morphological patterns of rodent tumours and increasing amounts of functional and mechanistic data in the field of carcinogenesis. It is considered that for the older studies conducted in the 1980s, the use of these

more modern criteria represents an improvement over the criteria used by the original study pathologist.

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 have been missed. This concern is highlighted, for example, by the fact that the PWG identified 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.

The data presented in the tables below are the findings reported in the original study reports. The results of the reanalysis by both BASF and the PWG are presented alongside the original study results in Appendix 1. Additional historical control data have also been collected by industry and are presented alongside the re-evaluation findings in Appendix 1. The historical control data presented in the tables below are those originally included in the study reports.

5.8.1 Carcinogenicity: oral

There are three carcinogenicity studies available in the rat and two studies available in the mouse.

5.8.1.1 Rat studies

Table 5.15 Carcinogenicity studies: Rat

Dose schedule [reference]	Dose levels	Observations and remarks (effects of major toxicological significance)

<table>
<tr>
<td>Daily in diet

Rat: Wistar

OECD 453

50/sex/group exposed for 2 years
10/ sex of controls and 20/sex of 8000 ppm exposed for 1 year

Krishnappa, 2002 in reference 2</td>
<td>0, 200, 2000 and 8000 ppm

Corresponds to 0, 9, 87 and 361 mg/kg/day in males
0, 12, 114 and 442 mg/kg/day in females</td>
<td>8000 ppm: 8/13 % ↓ food consumption (male/female), 11/10 % ↓ bodyweight by week 104 (male/female), 19/20 % ↑ absolute liver weight (male/female), 27/30 % ↑ in relative liver weight (male/female), 31 % ↑ absolute kidney weight (male), 41/24 % ↑ in relative kidney weight (male/female), 6 % ↓ haemoglobin (male), 6 % ↓ in haemocrit (male), 39/78 % ↑ in bilirubin (male/female), 300/242 % ↑ in gamma-glutamyl-transferase (male/female), ↑ hepatocyte hypertrophy (36/34in males/females compared to 0/1 in control), ↑ liver foci and masses in females (5/4 for foci/masses v 0/1 in control), ↑ incidence pale (9 males v 4 in control), cystic (7 males v 0 in control) and rough (25 male v 7 in control) kidneys,↑ bone marrow siderosis (10 female v 4 in control)

2000 ppm: 5 % ↓ food consumption (males), 30 % ↑ bilirubin (male), ↑ incidence rough kidneys (10 male v 7 in control)

200ppm: no significant effects.

Neoplastic findings (descendants and terminal (n=50))

The diagnostic criteria used by the study pathologists are those of the WHO and STP (Society of Toxicological Pathologists) nomenclature.

<table>
<tr><th colspan="9">Dose Level (ppm)</th></tr>
<tr><th colspan="4">Male</th><th colspan="4">Female</th></tr>
<tr><th>0</th><th>200</th><th>2000</th><th>8000</th><th>0</th><th>200</th><th>2000</th><th>8000</th></tr>
<tr><td colspan="9">Liver: adenoma</td></tr>
<tr><td>0</td><td>0</td><td>1(2%)</td><td>1(2%)</td><td>1(2%)</td><td>0</td><td>1(2%)</td><td>8(16%)</td></tr>
<tr><td colspan="9">Liver: carcinoma</td></tr>
<tr><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>2(4%)</td><td>1 (2%)</td></tr>
</table>

There were no increased tumour incidences in the thyroid or leydig cell in this study.

NOAEL of 8.5 (males) and 11.6 (males) mg/kg/day</td>
</tr>
<tr>
<td>Daily in diet

Rat: Sprague-Dawley

Non-guideline

2-years

50/sex/group exposed for 2 years
10/sex/group exposed for 1 year
15/sex/grp as satellite for blood sampling exposed for 2 years

Hunter B et al, 1983a in reference 2</td>
<td>0, 500, 2000 and 6000 ppm.

Corresponds to 18, 73 and 226 mg/kg/day in males 0, 21, 88, 272 mg/kg/day in females.</td>
<td>6000 ppm: 9/12 % ↓ food consumption (week 1-52: male/female), 25/36 % ↓ weight gain at 0-52 weeks (male/female), 32/42 % ↓ weight gain at 52-78 weeks (male/female), 41/40 % ↑ relative liver weight (male/female), 58/41 % ↑ relative kidney weight (male/female), 50 % ↑ blood urea nitrogen (male), Enlarged liver (32/17 in male/female v 7/7 in controls), ‘Ballooned’ hepatocytes (focal areas of hepatocyte degeneration) with periportal vacuolation (23 males v 12 in control), enlarged hepatocytes (8/11 in male/female v 0/1 in control), vacuolated hepatocytes 31/31 in male/female v 21/23 in control), kidney scarring (39/17 in male female v 23/14 in control), glomerulonephrosis (33/38 in male/female v 29/23 in control), Limiting ridge prominent in stomach (34/25 in male/female v 6/4 in control)

2000 ppm: 12/16 % ↓ weight gain at 0-52 weeks (male/female), 25/26 % ↓ weight gain at 52-78 weeks (male/female), 5/ 5 % ↓ food consumption (week 1-52: male/female), 18/37 % ↑ relative liver weight (male/female), 46/17 % ↑ relative liver weight (male/female), enlarged liver (16 male v 7 in control), ‘ballooned’ hepatocytes (20 males v 12 in control), vacuolated hepatocytes (34 female v 23 in control), enlarged hepatocytes (13 female v 0 in control), kidney scarring (31 male v 23 in control), limiting ridge prominent in stomach (17 male v 6 in control)

500 ppm: 12 % ↑ relative liver weight (male)</td>
</tr>
</table>

		Neoplastic findings (n=50) The diagnostic criteria used by the study pathologist are not documented in the study report. Dose level (ppm) Males: 0 | 500 | 2000 | 6000 Females: 0 | 500 | 2000 | 6000 **Thyroid parafollicular (c-cell) adenoma** 2 (4%) | 1 (2%) | 5(10%) | 5 (10%) | 1(2%) | 2 (4%) | 1(2%) | 1 (2%) [historical control range (males)] [0-2%; mean 0.3%] **Thyroid parafollicular (c-cell) carcinoma** 0 | 1 (2%) | 2 (4%) | 3 (6%) | 0 | 3 (6%) | 1 (2%) | 1 (2%) [historical control range (males)][0-18%; mean 12.9%] **Thyroid follicular adenoma** 0 | 1 (2%) | 2 (4%) | 4 (8%) | 1 (2%) | 0 | 1 (2%) | 1 (2%) [historical control range (males)][4-12%; mean 7.1%] **Thyroid follicular carcinoma** 0 | 0 | 0 | 1 (2%) | 0 | 0 | 1 (2%) | 1 (2%) [historical control range (males)] [0-8%; mean 2.0%] **Liver adenoma** 0 | 0 | 0 | 2 (4%) | 1 (2%) | 0 | 1 (2%) | 1 (2%) [historical control range (males)] [0-2%; mean 1.1%] **Liver carcinoma** 2 (4%) | 1 (2%) | 2 (4%) | 2 (4%) | 1 (2%) | 0 | 0 | 0 **Interstitial cell adenoma (leydig cells)** 1 (2%) | 1 (3%0 | 1 (2%) | 4 (7%) | | | | [historical control range (males)] [0-10%; mean 6.6 %] LOAEL = 18 (males) and 21 (females) mg/kg/day
Oral (diet) Rat: Sprague-Dawley Non-guideline - acceptable 2-years 50/sex/group exposed for 2 years 10/sex/group exposed for 1 year 15/sex/grp as satellite for blood sampling exposed for 2 years Hunter B et al, 1983b in reference 2	0, 100 ppm Corresponds to 0, 3 mg/kg/day in males 0, 4 mg/kg/day in females Test material purity: 93.6 and 95.3 % purity	No significant treatment related effects on bodyweight, haematology, clinical chemistry, organ weights or at necropsy ***Neoplastic findings*** There were no significant neoplastic findings observed in this study. These incidences have been provided because, as this study was conducted in the same laboratory, with the same strain of rats and under the same conditions as the study above (Hunter et al, 1983a), the identified control incidences are relevant to the evaluation of the neoplastic findings seen in the 1983a study.

Dose level (ppm)			
Males		Females	
0	100	0	100
Thyroid parafollicular (c-cell) adenoma			
1 (2 %)	2 (4 %)	1 (2 %)	3 (6 %)
Thyroid parafollicular (c-cell) carcinoma			
8 (16 %)	1 (2 %)	0	1 (2 %)
Thyroid follicular adenoma			
1 (2 %)	2 (4 %)	1 (2 %)	0
Thyroid follicular carcinoma			
2 (4 %)	0	1 (2 %)	0
Liver adenoma			
2 (4 %)	0	0	0
Liver carcinoma			
1 (2 %)	1 (2 %)	0	0
Interstitial cell adenoma (Leydig cells)			
0	2 (4 %)	-	-

NOAEL – 3 (males) and 4 (females) mg/kg/day

NB: The values for NOAEL and LOAEL are provided for information only: they have already been agreed at a PRAPER expert meeting

As shown in the table, in the three available rat studies, increased incidences of tumour findings were seen in the liver, thyroid and Leydig cells. A detailed analysis and discussion of these tumour findings is presented below.

Discussion

Liver

In Wistar rats, significant increases in adenomas and carcinomas were observed in females at the mid and high dose. Adenomas were also increased in males at the mid and high dose, but the incidences were very low (2 %). No historical control data were presented in the Pesticide Assessment Report. However, historical control data collected subsequently for the laboratory showed that the incidences of adenoma (at the high dose) and carcinoma (at the mid and high doses) were above that of the historical controls (see appendix 1). In Sprague-Dawley rats, only adenomas were slightly increased at the high dose (in males only). The incidence (4 %) was above the historical control range provided in the Pesticide Assessment Report, but is the same as that in the controls (4 %) of the second Hunter study initiated, in the same laboratory, six months after commencement of this study (see table 5.15). Therefore, the increase in adenomas in Sprague Dawley rats is not considered to be treatment related. Failure to see increased tumours in Sprague-Dawley rats is not inconsistent with the findings in Wistar rats as the top dose level employed in the Wistar study was higher (up to 8000 ppm compared with a top dose of 6000 ppm in Sprague-Dawley rats).

The PWG more or less confirmed the original findings. The only difference was a higher incidence of carcinomas in the top dose and a lower incidence of adenomas in the mid and top dose of the female Wistar rat. Following the PWG reanalysis, there was still evidence of an increased incidence of adenoma and carcinoma in female Wistar rats.

In a mechanistic study (see section 5.8.5), enzyme activity was measured in the livers of Wistar rats with the aim of investigating a phenobarbitone-like response. Consistent with phenobarbitone, Benzyloxyresorufin-O-debenzylase (BROD) activity was increased significantly (116-fold), whereas that of Ethoxyresorufin-O-deethylase (EROD) remained largely unaffected (2.4 fold increase) indicating induction of CYP450 of the 2B family. These findings were confirmed by the results of gene expression studies showing higher 2B mRNA levels after administration of Metazachlor (Nussler, 2010). Other findings consistent with a phenobarbitone-like response are the lack of genotoxicity and observations from repeat dose studies of increased liver weight and centrilobular hypertrophy. However, since several other substances are known to increase liver weight and/or induce centrilobular hypertrophy this evidence is not considered conclusive. 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. Therefore, based on current information, the mode of action for formation of these tumours remains unclear.

Overall, there is a clear carcinogenic effect in the liver of female Wistar rats (adenoma and carcinoma) of potential relevance to humans.

Thyroid

Parafollicular (C-cell) tumours

Increases in these tumours were observed in Sprague-Dawley, but not Wistar rats. The number of adenomas was slightly increased in males of the mid and high dose groups. The incidence was above the laboratory historical control range at both dose levels. In the treated males, there was also an increased incidence of carcinoma. However, as this was lower, even at the top dose, than the incidence observed in the control group (16 %) from the second Hunter study (initiated six months after this study in the same laboratory) the increase in carcinoma is not considered to be treatment related.

The number of adenomas identified by the PWG and internal BASF pathologists in the mid and high dose males was similar to that of the original study. However, the number of adenomas identified in the control and low dose groups was much higher upon re-examination – this annulled the difference between the controls and the treated groups. As this study in Sprague-Dawley rats was conducted in 1983, the PWG re-evaluation (which was based on more modern diagnostic criteria) is likely to provide a more accurate picture of the carcinogenic response produced by metazachlor. 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.

Concern for this tumour type is further reduced, however, as a similar tumour profile was not observed in the Wistar rat study, which employed higher doses.

Overall, therefore, taking all of this into account, it is considered unlikely that the very weak increase in tumour incidence observed in the parafollicular cells of the thyroid of Sprague-Dawley rats (i.e. a slight increase in benign adenomas in one sex and one strain) is a real, treatment-related effect of metazachlor.

Follicular tumours

Increases in these tumours (adenomas and carcinomas) were observed in Sprague-Dawley rats, but not in Wistar rats. The incidence was well within the historical control range and although a slight dose related increase in adenomas in males was observed, the dose response was nullified when the results from the second Hunter study (initiated in the same laboratory six months after this study) were included (see Appendix 1). The increase is, therefore, not considered treatment related. A slight increase in carcinomas was observed in top dose males and top and mid dose females. This increase was not only within the laboratory historical control range, but also lower than the incidence observed in the controls of the second Hunter study. Therefore, the carcinoma incidence is also considered not treatment related.

The PWG review found fewer adenomas in the mid dose group, annulling the dose response, but a higher incidence in the high dose group. As all incidences were still within the historical control range, their review is not considered to affect the conclusion.

The mechanistic investigations on the effect of metazachlor on thyroid hormone homeostasis add very little useful information.

Similar tumour findings were not observed in Wistar rats, although these animals were exposed to much higher dose levels.

Overall, the marginal dose related increases in adenoma and carcinoma, which were well within the historical control range, are not considered to be treatment related.

Testis

Interstitial cells (Leydig cells)

Increases in these tumours were observed in Sprague-Dawley rats, but not in Wistar rats. A significant increase in adenoma was observed at the high dose. This increased incidence was within the laboratory historical control range and is, therefore, not considered treatment related. The results were confirmed by the PWG - the only difference was that a slightly higher incidence of adenomas was observed in the control group.

Summary of the 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 (adenomas and carcinomas). All other tumours observed are considered unlikely to be treatment related.

5.8.1.2 Mouse studies

Table 5.16 Carcinogenicity studies: mice

Dose schedule [reference]	Dose levels	Observations and remarks (effects of major toxicological significance)
Daily in the diet Swiss OECD 453 18 month 50/sex/group	0, 100, 1000 or 4000 ppm Corresponds to 15, 154 and 578 mg/kg/day in males 0, 16, 163 and 640 mg/kg/day	4000 ppm: ↓ food consumption (male/female), 5-13 % ↓ bodyweight in males up until week 13, 8-10 % ↓ bodyweight in females up until week 52, 30 % ↑ absolute kidney weight (male), 27 % ↑ in relative kidney weight (male), 19 % ↓ neutrophils (female), ‚Bladder epithelial hyperplasia (1 male) 1000 ppm: 4-9 % ↓ bodyweight in females up until week 52, 33% ↑ in relative kidney weight (male)

<table>
<tr>
<td>Kumar, 2003 in reference 2</td>
<td>in females

97.7% purity</td>
<td>100 ppm: no adverse effects

Neoplastic findings
The diagnostic criteria used by the study pathologist are those of the WHO and STP nomenclature.

<table>
<tr><td colspan="9">Dose level (ppm)</td></tr>
<tr><td colspan="4">males</td><td colspan="4">females</td></tr>
<tr><td>0</td><td>100</td><td>1000</td><td>4000</td><td>0</td><td>100</td><td>1000</td><td>4000</td></tr>
<tr><td colspan="9">Bladder: epithelial hyperplasia</td></tr>
<tr><td>0</td><td>0</td><td>0</td><td>1 (2%)</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td colspan="9">Transitional cell papilloma</td></tr>
<tr><td>0</td><td>0</td><td>1 (2%)</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td colspan="9">Transitional cell carcinoma</td></tr>
<tr><td>0</td><td>0</td><td>0</td><td>1 (2%)</td><td>0</td><td>0</td><td>0</td><td>2 (4%)</td></tr>
</table>

There were no increased incidences observed in the liver, kidney or lymphoreticular system.

NOAEL of 154 (males) and 163 (females) mg/kg/day</td>
</tr>
<tr>
<td>Daily in the diet

CD-1

US-EPA

2-year

50/sex/group exposed for 2 years
10/sex/group exposed for 1 year

Barnard et al, 1983 in reference 2</td>
<td>0, 200, 700 or 2500 ppm

Corresponds to 19, 72, and 252 mg/kg/day in males
0, 21, 74 and 273 mg/kg/day in females

Purity 93.6 – 95.3 %</td>
<td>2500 ppm: 22 % ↓ bodyweight gain (female), 18/13 % ↑ absolute liver weight (male/female) at 52 but not 104 weeks,
Focal and diffuse hyperplasia bladder (35/17 in male/female v 6/2 in control), nuclear enlargement bladder (16/9 in male/female v 5/2 in control)

700 ppm: 26/18 % ↑ absolute liver weight (male/female) at 52 but not 104 weeks,

200 ppm: no adverse effects

Neoplastic findings: descendants and week 104 (n=50)

The diagnostic criteria used by the study pathologist are not described in the study report

<table>
<tr><td colspan="9">Dose level (ppm)</td></tr>
<tr><td colspan="4">males</td><td colspan="4">females</td></tr>
<tr><td>0</td><td>200</td><td>700</td><td>2500</td><td>0</td><td>200</td><td>700</td><td>2500</td></tr>
<tr><td colspan="9">Lymphocytic leukaemia</td></tr>
<tr><td>0</td><td>0</td><td>0</td><td>2(4%)</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td colspan="9">Kidney cortical (renal tubule) adenoma</td></tr>
<tr><td>0</td><td>1 (2%)</td><td>3 (6%)</td><td>3(6%)</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td colspan="9">Kidney cortical (renal tubule) carcinoma</td></tr>
<tr><td>0</td><td>0</td><td>1 (2%)</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td colspan="9">Liver adenoma</td></tr>
<tr><td>11 (22%)</td><td>10 (20%)</td><td>8 (16%)</td><td>13(26%)</td><td>0</td><td>0</td><td>1 (2%)</td><td>3 (6%)</td></tr>
<tr><td colspan="9">Liver carcinoma</td></tr>
<tr><td>11 (22%)</td><td>15 (30%)</td><td>15 (30%)</td><td>12 (24%)</td><td>0</td><td>1 (2%)</td><td>0</td><td>1 (2%)</td></tr>
</table>
There were no increased tumour incidences in the bladder.

A NOAEL of 72 (males) and 74 mg/kg/day in females</td>
</tr>
</table>

NB: The values for NOAEL and LOAEL are provided for information only: they have already been agreed at a PRAPER expert meeting

As shown in the table, in the two available mouse studies an increased tumour incidence was observed in the bladder, lymphoreticular system, kidney and liver. A reanalysis of the tumour findings was also performed on the mouse sections from both studies (see Appendix I).

Discussion

Bladder

In Swiss (but not CD-1) mice, a small increase in bladder transitional cell carcinoma was observed in top dose males and females. Bladder (transitional cell) carcinoma had never been recorded by this laboratory in previous carcinogenicity studies. Re-examination by the BASF internal pathologists and the PWG failed to confirm the original results. Re-analysis by both led to the reclassification of the carcinomas as (transitional cell) papillomas. In addition, a (transitional cell) papilloma was found in the control group in males and in low dose females. Overall, the re-analysis revealed that there were no (transitional cell) carcinomas in either sex and that there were no significant differences between the treated groups and controls in the incidence of the (transitional cell) papillomas in males. In females, although there was an increase (4 %) in papillomas at the top dose of 4000 ppm, the lack of dose response indicated that this was not treatment related as one tumour was observed at the low dose (100 ppm) with none at a dose ten times higher (1000 ppm).

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.

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.

Overall, the weak increase in tumour incidence in the bladder of Swiss mice, if any, is regarded a chance finding unrelated to treatment.

Lymphocytic (lymphoblastic) Leukaemia

In CD-1 (but not Swiss) mice, a small increase in lymphoblastic leukaemia was observed in high dose males only. These tumours are rare (out of 46 studies (total of 2565 mice) it was observed in 3 animals from two studies). The PWG re-classified them as malignant lymphomas (a form of tumour of the lymphoreticular system, which are very common in mice) since no obvious indication of leukaemia was noted in the peripheral blood smears and found a similar incidence in the controls. As this study was conducted in 1983, the PWG re-evaluation is likely to provide a more accurate picture of the carcinogenic response produced by metazachlor. Furthermore, their explanation of the re-classification of the leukaemia is sound and coherent. Therefore, the re-classification of the findings is accepted.

Concern is further reduced as similar findings were not observed in Swiss mice, although these animals were exposed to a higher dose level.

Overall, it is unlikely that the weak increase in tumour incidence in the lymphoreticular system, if any, of CD-1 mice (observed in males only) is treatment related.

Kidney

In CD-1 (but not Swiss) mice, small increases in renal tubule adenomas were observed in males. A single renal tubule carcinoma was identified in mid dose males. No historical control data were provided in the original study report. 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. 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.

Similar findings were not observed in Swiss mice, although these animals were exposed to a higher dose level.

Re-examination of the kidney slides (see section 5.8.5) did not reveal any evidence of sustained toxicity or regeneration, raising a question about the origin of the observed tumours. In addition, no relevant kidney effects were observed in either the chronic or 28-day studies (conducted on CD-1 mice), nor was kidney enzyme activity found to be increased in the 14-day mechanistic study (see table 5.17).

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.

Liver

In CD-1 (but not Swiss) mice, a weak, dose-related, increase in adenomas was observed in females. A single incidence of carcinoma was detected in the low and high dose groups. No historical control data were included within the original study report. However, historical control data for this laboratory, presented alongside the PWG findings, suggest that both the adenoma and carcinoma incidences are within the historical control ranges. Although the increase in adenoma incidence, reported in the original study, was dose-related, it was well within the historical control range and similar findings were not observed in Swiss mice, exposed to much higher doses. Therefore, this increase is considered a chance finding unrelated to treatment. The PWG more or less confirmed the original study findings.

Overall, the increase in adenomas and carcinomas observed in female CD-1 mice was not related to treatment with metazachlor.

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. All other tumours are considered unlikely to be treatment related.

5.8.2 Carcinogenicity: inhalation

No data

5.8.3 Carcinogenicity: dermal

No data

5.8.4 Carcinogenicity: human data

No data

5.8.5 Other relevant information

A number of mechanistic studies have been conducted to elucidate the carcinogenic potential of metazachlor (only draft reports have been received so far). These are summarised below.

Table 5.17 Additional information relevant to carcinogenicity

Dose schedule [reference]	Dose levels	Observations and remarks (effects of major toxicological significance)
Microcrystallisation in the urinary bladder and enzyme induction in the liver and kidney of rat 14-day study Oral (diet) Wistar Rat Buesen *et al*, 2009a	0 and 8000 ppm 550 mg/kg/day in males 500 mg/g/day in females	Aim Study aimed at investigating the presence of micro-crystals in the urine as a potential mode of action in metazachlor- induced bladder carcinogenesis. Also aimed at investigating enzyme induction in the liver and kidneys. Results No evidence of microcrystallisation was observed Bodyweight was significantly decreased in males (5 %) Liver Increased absolute liver weight was observed in females (26 %). Increased relative liver weights in both sexes (16/26 % in male/females). Relevant changes in enzyme activity in females: - Cytochrome P450 (Cyt.P450) – 2.1 fold increase - Ethoxyresorufin-O-deethylase (EROD) -2.4 fold increase - Benzoxyresorufin-O-debenzylase (BROD) – 116 fold - 4-Methylumbeliferone-glucuronyltransferase (MUF-GT) – 1.3 fold Kidney: Increases absolute kidney weight in females (13 %) and relative kidney weight in both sexes (12/16 in males/females). Relevant changes in enzyme activity in females: -Cytochrome P450 (Cyt.P450) –increase not measurable as non detected in controls -Ethoxyresorufin-O-deethylase (EROD) -1.7 fold increase -Benzyloxyresorufin-O-debenzylase (BROD) – 6 fold increase -4-Hydroxybiphenyl-glucuronyltransferase (HOBI-GT) – 24 fold increase Conclusion Microcrystallisation is not involved in the mode of action leading to bladder tumour development with metazachlor. Administration of metazachlor resulted in an increase in BROD, but not EROD activity in both the liver and kidney.
mRNA Analysis of	500 ppm	Aim

Liver tissue form Rat treated for 3 and 7 days with Phenobarbitone or BAS479H (metazachlor) Nussler, 2010	Phenobarbitone and 8000 ppm Metazachlor PCR – 35 cycles Expression measured using densitometry of signals on the gel	To investigate the mRNA levels of enzymes involved in phase I and II drug metabolism by <u>semi-quantitative</u> RT-PCR in liver tissue of rats Results As the method employed was semi-quantitative PCR the results below are only indicative of changes in gene expression The effect of metazachlor (M) and phenobarbitone (PB) on gene expression of: <u>Cytochrome P450 Iso-forms</u> CYP2B1 - Induction by both substances on day 3 and 7 CY2B2 – Induction by both substances on day 7 CYP2C6 and CYP2C11 – signals to weak for analysis <u>Phase II Drug metabolizing Enzymes</u> UDP- glucuronosyltransferase 1A1 – Weak induction by PB on day 3 (but not day 7) and by M on day 7 UDP-glucuronosyltransferase 1A6- induction by M on day 3 and 7 Glutathione- S- transferase A1 – very slight induction by PB on day 3 Glutathione- s-transferase- A2 – very slight increase by PB on day 3 and 7 Multidrug resistance programme 2 – very slight induction by PB on day 7 Matrix metalloproteinase 2 – slight induction by PB on day 7 and M on day 3 Conclusion Metazachlor and phenobarbitone increase the mRNA levels of certain cytochrome P450 iso-forms similarly, whereas differences were more pronounced for phase II metabolising enzymes
Thyroid hormone study 28-day study Wistar rats Oral (diet) Bueson et al, 2009b	0 and 8000 ppm 500 mg/kg/day in males 650 mg/kg/day in females	Aim Study aimed at showing a phenobarbitone-like response in the thyroid. Results A 1.5 fold increase in TSH in males was observed accompanied by a minimal to slight increase in follicular hypertrophy/hyperplasia. Both T4 and T3 were within the expected ranges. Conclusion Failure to see any changes in T3 or T4 levels questions the hypothesis that the mode of action is the same as that of phenobarbitone.
Thyroid perchlorate discharge assay Wistar rats 28-day study Oral (diet) Buesen *et al* , 2009c	Metazachlor: 974 mg/kg/day 6 males/group	Aim Study aimed at investigating thyroid specific toxicity, as a potential mechanism in metazachlor- induced thyroid carcinogenesis. Results Metazachlor does not perturb thyroid hormone homeostasis by inhibition of thyroid peroxidase, the enzyme that liberates iodine for addition onto thyroglobulin and production of T3 and T4. Conclusion This indicates that thyroid peroxidase inhibition, a mechanism of thyroid carcinogenesis relevant to humans, is not involved in thyroid tumour development with metazachlor
Re-examination of renal histopathology in carcinogenicity studies of metazachlor in mice Hard, 2009		Aim Study aimed at determining whether a treatment related toxicological mode of action based on sustained toxicity and/or regeneration could be established. Results Re-examination of the kidney did not reveal any evidence of sustained toxicity or regeneration. Conclusion This indicates that the kidney tumours were unlikely to have arisen through this mechanism.

Microcrystallisation in the urinary bladder and enzyme induction in the liver and kidney of mice 14-day study Oral (diet) CD-1 mice Buesen *et al*, 2009d	0 and 8000 ppm 1400 mg/kg/day in males 1900 mg/g/day in females	Aim Study aimed at investigating the presence of micro-crystals in the urine or as a potential mode of action in metazachlor- induced bladder urinary-tract carcinogenesis. Also aimed at investigating enzyme induction in the liver and kidney. Result No evidence of microcrystallisation was observed Terminal bodyweight was decreased in both males (12 %) and females (4 %) Liver A statistically significant increase in relative liver weight was observed in females (18 %). Relevant changes in enzyme activity in females: - Cytochrome P450 (Cyt.P450) – 2.1 fold increase - Ethoxyresorufin-O-deethylase (EROD) - 4.4 fold increase - Pentoxyresorufin-O-depentylase (PROD) – 9.9 fold - Benzyloxyresorufin-O-debenzylase (BROD) – 11.2 fold - 4-Methylumbeliferone-glucuronyltransferase (MUF-GT) – 1.7 fold - 4-Hydroxybiphenyl-glucuronyltransferase (HOBI-GT) – 2.1 fold increase Kidney A statistically significant increase in relative kidney weight was observed in males (18 %). This was considered secondary to the decrease in bodyweight. - 4-Methylumbeliferone-glucuronyltransferase (MUF-GT) – 1.4 – 1.5 fold in males and females The activity of all other enzymes was below the level of detection in the control animals. Although some activity could be detected in treated animals, a clear induction could not be proven. Conclusion Microcrystallisation is not involved in the mode of action leading to bladder tumour development with metazachlor. Administration of metazachlor did not result in an enzyme induction profile in the liver similar to that observed with phenobarbitone. No significant changes in enzyme induction were noted in the kidney
S-Phase Response Study in MF1 mice; HsdOla: MF1 mice Three different treatment periods of 7, 28 and 90 days were used Kumar S, 2009	0, 2500 and 4000 ppm S-phase was measured using BrdU-stained cells Apoptosis detected by TUNEL assay	Aim Study aimed at determining whether metazachlor induces cell proliferation in the bladder as a potential mode of action for metazachlor-induced bladder carcinogenesis. Results A significant increase in S-phase response was observed in the urinary bladder in both sexes of mice. The extent of this effect was both time and dose dependent. A slight increase was observed in the 2500 ppm group (the increase was significant after 90 days). The S-phase response was significantly increased at all time points in 4000 ppm treated mice (except in female mice on day 28). In general, males were slightly more affected than females. Apoptosis was increased in all treated animals, with animals most affected after 90-days in the 2500 and 4000 ppm treatment groups. Conclusion See summary assessment below
S-Phase Response Study in CD-1 mice;	0, 200, 700, 2500 and 4000 ppm	Aim Study aimed at determining whether metazachlor induces cell proliferation in the bladder as a potential mode of action for metazachlor-induced bladder

CD-1 mice Three different treatment periods of 7, 28 and 90 days were used S-phase was measured using BrdU-stained cells Apoptosis detected by TUNEL assay Kaspers, 2009	approximately equivalent to 26-30, 90-113, 408-452 and 643-779 mg/kg/day in males and 33-36, 114-147, 475-541 and 785-1026 mg/kg/day in females.	carcinogenesis. Results A significant increase in cell proliferation was observed in the urinary bladder in both sexes of mice. The extent of this effect was both time and dose dependent - no significant increase was observed in any group at day 7 and the 200 ppm dose group did not show any clear increase in males and no increase was observed in females. Apoptosis was more often observed with an increase of S-phase response, especially in the 2500 ppm group. In concordance with the increased cell proliferation in urinary bladder a dose related increase in diffuse hyperplasia of the transitional epithelium was observed ≥ 2500 ppm. Cell proliferation and diffuse hyperplasia was more prevalent in males than females. Conclusion See summary assessment below.
Summary Assessment		Comparison of the S-phase results from CD-1 and MF1 mice show that metazachlor leads to a dose related increase in cell proliferation in both strains, although the proliferative response was more pronounced in CD-1 mice than MF-1 mice.

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. For the other tumour types no clear modes of action were identified.

5.8.6 Summary and discussion of carcinogenicity

The carcinogenicity of metazachlor has been investigated in rats (Wistar and Sprague-Dawley) and mice (Swiss and CD-1).

In the rat, metazachlor was shown to have a clear carcinogenic effect in the liver (adenomas and carcinomas).

In the mouse, metazachlor appeared to have a weak carcinogenic effect in the kidney. In the kidney only benign tumours were observed and the effect was inconsistent between strains and sexes.

On the basis of these findings in animals, classification for carcinogenicity is justified.

In accordance with the criteria in Directive 67/548/EEC, classification in category 1 for carcinogenicity is not justified given that there is no evidence of metazachlor having caused cancer in humans. It is therefore necessary to decide whether to classify metazachlor in category 2 or category 3.

Since increased tumours have been seen in 2 species, a simple argument for category 2 classification can be made. 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. It is therefore possible that the kidney findings in the mouse are chance observations.

In view of these considerations, the available evidence is deemed to best match the criteria for classification as a category 3 carcinogen.

Similarly, according to Regulation EC/1272/2008, classification as a category 2 carcinogen is judged to be appropriate. There are no grounds to draw attention to a particular route of exposure on the label.

Directive 67/548/EEC criteria: propose Carc. Cat 3; R40

Regulation EC/1272/2008: propose Carc 2; H351

5.8.7 Toxicity for reproduction

5.8.8 Effects on fertility

Fertility has been investigated in a three-generation and a two-generation study.

Table 5.18 Fertility studies

<table>
<tr><th>Method

Species</th><th>Exposure conditions, & doses</th><th>Observations and remarks</th></tr>
<tr><td>3-generation study

OECD 416

Wistar rats

30/sex/group

GLP

Ganiger, 1999</td><td>Oral, diet

0, 200, 2000 or 8000 ppm

F0: Equivalent to 16, 151 and 661 mg/kg/day in males and 20, 203 and 806 mg/kg/day in females

F1: Equivalent to 16, 171 and 743 mg/kg/day in females and 20, 202 and 841 mg/kg/day in females

F2: equivalent to 15, 153 and 662 mg/kg/day in males and 20, 192 and 760 mg/kg/day in females</td><td>Parental toxicity
Bodyweight and food consumption
8000 ppm: Bodyweight was reduced at the time of mating in the F0 (8/7 % in males/females), F1 (17/9 % in males/females) and the F2 (15/12 % in males/females) generation.

Female bodyweight and food consumption was also decreased throughout gestation and lactation at 8000 ppm and decreases were also observed in the 2000ppm group at the beginning of gestation and lactation

<table>
<tr><th></th><th>Bodyweight</th><th>0</th><th>200</th><th>2000</th><th>8000</th></tr>
<tr><td>F0</td><td>Gestation Day 0 (g)</td><td>211</td><td>207</td><td>200*</td><td>198*</td></tr>
<tr><td></td><td>Gestation Day 20 (g)</td><td>321</td><td>315</td><td>306</td><td>286*</td></tr>
<tr><td></td><td>Food (g/d)</td><td>21.2</td><td>20.9</td><td>21.5</td><td>20.2</td></tr>
<tr><td></td><td>Lactation Day 0 (g)</td><td>239</td><td>232</td><td>226*</td><td>215*</td></tr>
<tr><td></td><td>Lactation Day 21 (g)</td><td>272</td><td>271</td><td>266</td><td>243*</td></tr>
<tr><td></td><td>Food (g/d)</td><td>44.4</td><td>42.8</td><td>42.3</td><td>37.4*</td></tr>
<tr><td>F1</td><td>Gestation Day 0 (g)</td><td>225</td><td>224</td><td>227</td><td>196*</td></tr>
<tr><td></td><td>Gestation Day 20 (g)</td><td>338</td><td>337</td><td>333</td><td>276*</td></tr>
<tr><td></td><td>Food (g/d)</td><td>23.8</td><td>23.9</td><td>22.7</td><td>20.5*</td></tr>
<tr><td></td><td>Lactation Day 0 (g)</td><td>256</td><td>256</td><td>256</td><td>218*</td></tr>
<tr><td></td><td>Lactation Day 21 (g)</td><td>277</td><td>285</td><td>284</td><td>246*</td></tr>
<tr><td></td><td>Food (g/d)</td><td>43.5</td><td>43.2</td><td>45.1</td><td>34.9*</td></tr>
<tr><td>F2</td><td>Gestation Day 0 (g)</td><td>234</td><td>229</td><td>216*</td><td>193*</td></tr>
<tr><td></td><td>Gestation Day 20 (g)</td><td>342</td><td>336</td><td>324</td><td>278*</td></tr>
<tr><td></td><td>Food (g/d)</td><td>23.1</td><td>22.3</td><td>22.0</td><td>18.1</td></tr>
<tr><td></td><td>Lactation Day 0 (g)</td><td>263</td><td>256</td><td>247*</td><td>217*</td></tr>
<tr><td></td><td>Lactation Day 21 (g)</td><td>284</td><td>282</td><td>276</td><td>249*</td></tr>
<tr><td></td><td>Food (g/d)</td><td>48.8</td><td>50.5</td><td>43.7</td><td>36.0*</td></tr>
</table>

Fertility effects
No effect on mating performance or number of pregnancies was observed in any treatment group in any generation. However, the</td></tr>
</table>

<table>
<tr><td></td><td></td><td>numbers of corpora lutea and implantation sites were significantly decreased in the F1 and F2 generations at 8000 ppm.

<table>
<tr><th colspan="2"></th><th>0</th><th>200</th><th>2000</th><th>8000</th></tr>
<tr><td>F0</td><td>Corpora lutea (#)</td><td>14.4</td><td>14.5</td><td>13.9</td><td>13.6</td></tr>
<tr><td></td><td>Implantations (#)</td><td>13.0</td><td>13.2</td><td>11.9</td><td>11.8</td></tr>
<tr><td></td><td>Pre-implantation loss (%)</td><td>9.7</td><td>9.3</td><td>14.7*</td><td>13.4</td></tr>
<tr><td>F1</td><td>Corpora lutea (#)</td><td>14.8</td><td>14.7</td><td>14.8</td><td>11.8*</td></tr>
<tr><td></td><td>Implantations (#)</td><td>13.2</td><td>12.8</td><td>13.3</td><td>10.3*</td></tr>
<tr><td></td><td>Pre-implantation loss (%)</td><td>10.8</td><td>12.5</td><td>10.2</td><td>12.2</td></tr>
<tr><td>F2</td><td>Corpora lutea (#)</td><td>14.1</td><td>14.8</td><td>14.0</td><td>12.3*</td></tr>
<tr><td></td><td>Implantations (#)</td><td>13.2</td><td>14.1</td><td>13.2</td><td>11.1*</td></tr>
<tr><td></td><td>Pre-implantation loss (%)</td><td>6.8</td><td>4.9</td><td>5.9</td><td>10.1</td></tr>
</table>

Offspring effects
F1: At 8000 ppm, the number of dead pups was higher (14 v 3 in the controls). Pup weight was lower from day 4 of lactation (8 %) and remained lower throughout lactation (26% on day 21).

No effects were observed at any other dose level

F2: At 8000 ppm, litter size was smaller (9.6 v 12.5 in the control). Pup survival index was lower at all time points during lactation and was reduced by 14 % by day 21. Pup weight was lower on day 4 of lactation (14 %) and remained lower throughout lactation (24% by day 21).

No effects were observed at any other dose level

F3: 8000 ppm, litter size was smaller (10.1 v 12.6 in controls). Survival index was lower from day 4 of lactation (3 % reaching 10 % by day 21). Pup weight was lower from day 4 of lactation (10 %) and remained lower throughout lactation (25 % by day 21).

2000 ppm: A slight reduction in the survival index was observed on day 14 of lactation (2-4 %). Pup weight was lower from day 4 (9%) and remained lower throughout lactation (9 % on day 21).

Reproductive NOAEL of 151 (males) and 192 (females) mg/kg/day
Offspring NOAEL of 16 (males) and 15 (females) mg/kg/day in both sexes</td></tr>
<tr><td>2-generation study (borderline acceptable)

EPA

Sprague-Dawley

25/sex/group

GLP

Cozens et al, 1982</td><td>Oral, diet

0, 10, 100 or 1000 ppm

Equivalent to 1, 8, 79 mg/kg/day in m and 1, 9 and 97 mg/kg/day in f</td><td>Parental toxicity
F0: The number of animals not producing litters was high in all groups (8, 4, 6, 3 in control, 10, 100 and 1000 ppm, respectively). Vaginal smears from the majority of these animals did not reveal any evidence of mating. Histopathology of males did not reveal any treatment related findings. No effects on bodyweight or food consumption were observed.

F1: Again, the number of females not producing litters was high in all groups (7, 5, 6, 5 in control, 10, 100 and 1000 ppm, respectively). Vaginal smears indicated that a proportion of these animals had mated. At 1000 ppm, initial female pre-mating gain during week 5-9 was significantly lower than controls (11 %). No effects on bodyweight were observed throughout gestation or lactation.

Offspring effects
F1: No adverse treatment related effects were observed.
F2: No adverse treatment related effects were observed.</td></tr>
</table>

		NOAEL 1000 ppm (79 (males) and 97 (females) mg/kg/day)

NB: The values for NOAEL and LOAEL are provided for information only: they have already been agreed at a PRAPER expert meeting

The effects of metazachlor on fertility have been investigated in a three generation study and a two generation study in rats.

In the three generation study, bodyweights were statistically significantly lower in all generations at 8000 ppm. No effect on mating performance or the number of pregnant animals was observed. Smaller litter sizes in top dose groups of the F2 and F3 generations were observed and this finding was associated with lower numbers of corpora lutea and implantations in the parental females from these groups. Several effects on offspring were also noted in the top dose group (reduced survival index and lower pup weight). Due to the extent of the general toxicity observed at this dose (bodyweight was reduced by 9 % and 12 % at time of mating in the F1 and F2 generations, respectively), it is likely that these effects were a secondary, non specific consequence of maternal toxicity and not a specific effect on reproduction.

In the two generation study, no treatment related effects were observed. However, the value of this study is compromised by a poor mating performance in all treatment groups, including the controls.

Overall, the results suggest that metazachlor does not affect fertility and reproductive performance.

5.8.9 Developmental toxicity

Developmental toxicity has been investigated in studies conducted in rats and rabbits.

5.8.9.1 Rats

Table 5.19 Developmental studies: Rat

Method Species	Exposure conditions	Doses	Observations and remarks
Developmental toxicity Non-guideline (acceptable) Rat (Sprague Dawley) 20/group Cozens et al, 1980a	Oral (gavage) Vehicle: aq. Carboxy-methylcellulose Days 6-15 of gestation	0, 50, 150 or 450 mg/kg/day	Maternal toxicity: No maternal deaths occurred. Signs of toxicity (increased salivation, piloerection, unkempt appearance and lethargy) were observed at the top dose level; increased salivation was also observed at 150 mg/kg/day. Weight gain at the top dose was marginally lower during the first four days; however, weight gains over treatment and study periods were comparable in all groups. Developmental effects: No treatment-related effects were observed regarding litter parameters Foetal findings were limited to a single major malformation (interventricular septal defect) noted in one pup at 450 mg/kg/day; the incidence was within the historical control range. No other effects showed any relation to treatment, although the level of detail reported was low. Maternal NOAEL of 50 mg/kg/day. Foetal NOAEL of 450 mg/kg/day
Developmental Toxicity	Oral (gavage) Vehicle:	0, 50, 250 or 500 mg/kg/day	Maternal toxicity: No maternal deaths occurred. Signs of toxicity (dullness, nasal discharge and wet perineum) were noted at 500 mg/kg/day; nasal discharge was also observed at

<table>
<tr>
<td>OECD 414 (1981)

Rat (Wistar)

27/ group

Ponnana, 1996c</td>
<td>Peanut oil

Days 6-15 gestation</td>
<td></td>
<td>250 mg/kg/day. Mean bodyweights at the top dose were lower on days 15 (8 %) and 20 (6 %). Food consumption (16 %) and weight gain (71 %) were lower throughout the treatment period in top dose females.

Developmental effects:, At 500 mg/kg/day pup weight was reduced and there was an increase of small foetuses and renal pelvis dilatation (see table below), effects indicative of a slight retardation in development. Additionally, there was evidence of a slight retardation of ossification at the top dose level.

<table>
<tr><th rowspan="2">Parameter / time point</th><th colspan="4">Dose level (mg/kg bw/d)</th></tr>
<tr><th>0</th><th>50</th><th>250</th><th>500</th></tr>
<tr><td>Pup weight (g)</td><td>3.6</td><td>3.5</td><td>3.6</td><td>3.4*</td></tr>
<tr><td colspan="5">External findings (per no of foetuses)</td></tr>
<tr><td>Small foetus (%)</td><td>0</td><td>0</td><td>0</td><td>4.7</td></tr>
<tr><td>Haematoma (%)</td><td>0</td><td>0</td><td>0</td><td>0.4[a]</td></tr>
<tr><td>Spine bifida (%)</td><td>0</td><td>0</td><td>0</td><td>0.4[a]</td></tr>
<tr><td>Anal atresia (%)</td><td>0</td><td>0</td><td>0</td><td>0.4[a]</td></tr>
<tr><td>Rudimentary tail (%)</td><td>0</td><td>0</td><td>0</td><td>0.4[a]</td></tr>
<tr><td colspan="5">Visceral findings</td></tr>
<tr><td>Renal pelvis dilatation (%)</td><td>1.3</td><td>3.1</td><td>1.6</td><td>6.0*</td></tr>
<tr><td colspan="5">Skeletal findings</td></tr>
<tr><td>Sternebrae 4/5: fused (%)</td><td>0</td><td>0</td><td>0</td><td>1.7</td></tr>
</table>
*significantly different to controls (p<0.05)[Dunnett's test]
[a]observations in a single foetus

Maternal NOAEL of 250 mg/kg/day
Developmental NOAEL of 250 mg/kg/day</td>
</tr>
<tr>
<td>Developmental toxicity

OECD 414

Rat (Wistar)

28/group

Yogeesh, 2002</td>
<td>Oral (gavage)

Vehicle: aq. Carboxy-methylcellulose

Days 6 -19 of gestation</td>
<td>0, 100, 250 or 500 mg/kg/day</td>
<td>Maternal toxicity: no maternal deaths occurred. Signs of toxicity (dullness, weakness, lethargy and nasal discharge) were observed throughout the treatment period at 500 mg/kg/day. Bodyweight was decreased at this dose (12 %), as was bodyweight gain (42 %) and food consumption (23%) throughout the treatment period. No effects were observed at other doses.

Developmental effects: At 500 mg/kg/day pup weight was reduced and there was evidence of a slight retardation of ossification at the top dose level. Of the large number of morphological parameters assessed in the foetal examination there were a very small number of inter-group differences, but there was no consistent pattern suggesting a treatment-related effect (see table below). These isolated changes are minor in nature.
No major malformations were observed.

Maternal NOAEL of 250 mg/kg/day</td>
</tr>
</table>

			Developmental NOAEL of 250 mg/kg/day **Dose level** (mg/kg bw/d) **0** / **100** / **250** / **500** **Foetal findings** (% foetal incidence [% litter incidence]) **Foetal weight** (g): 3.6 / 3.6 / 3.5 / 3.1** **External malformations total**: - / 0.3 [3.8] / - / 0.8 [9.1] **Limb flexure**: - / - / - / 0.8 [9.1] **Distended bladder**: - / 0.65 / 0.79 / 4.07* **2nd sternebra hypoplastic**: 0.75 / - / 7.32* / 5.74* **Pubis hypoplastic**: - / - / - / 3.28 **5th sternebra split**: - / - / - / 0.82 **Thoracic vertebra asymmetric**: - / - / - / 4.92* **Forelimb flexure**: - / - / - / 2.46

NB: The values for NOAEL and LOAEL are provided for information only: they have already been agreed at a PRAPER expert meeting

The developmental toxicity study has been investigated in three studies in rat.

In the earliest study, no marked maternal toxicity or any treatment related developmental findings were observed. In the subsequent two studies, marked maternal toxicity was observed at the top doses manifested as a marked reduction in body weight gain over the treatment period (42- 71 %). No malformations likely to be of concern were noted in either study and the developmental effects observed were considered to be a non specific consequence of the maternal toxicity and not a direct effect on development.

Overall, the results suggest that metazachlor does not cause specific developmental toxicity in rats.

5.8.9.2 Rabbit

Table 5.20 Developmental studies: Rabbit

Method Species	Exposure conditions	Doses	Observations and remarks
Developmental Toxicity EPA (1978) Rabbit (Himalayan) 14-15/group Zeller & Merkle, 1980	Oral (gavage) Vehicle: aq. Carboxy-methylcellulose Days 6-18 of gestation Animals sacrificed on day 29	0, 25, 50 or 100 mg/kg/day	Maternal toxicity: No deaths or signs of toxicity were observed in this study. Slight weight loss was observed at the top dose during the early part of the treatment (day 6-7 -1.33 g v 21.20 g at 100 mg/kg/day v controls) and was accompanied by a reduction in food consumption at this dose (17.5 %). However, weight gain over the whole period was comparable in all groups and no significant bodyweight effects were observed. Developmental toxicity: There were no effects observed suggestive of developmental toxicity Maternal NOAEL of 50 mg/kg/day Foetal NOAEL of 100 mg/kg/day (top dose tested).
Developmental toxicity	Oral (gavage) Vehicle: aq.	0, 250 or 750 mg/kg/day	Maternal toxicity: Two treatment related deaths were observed at 750 mg/kg/day. Weight loss was observed throughout dosing at 750

<table>
<tr>
<td>EPA guideline (1978)

Rabbit (Himalayan)

15/group

Hildebrand & Merkle, 1981</td>
<td>Carboxy-methylcellulose

Days 6-18 of gestation

Animals sacrificed on day 29</td>
<td></td>
<td>mg/kg/day (days 6-19: -371.5 g v 30.4 g in the top dose group and controls, respectively). Food consumption was also decreased throughout treatment and then higher thereafter. No adverse effects were observed at 250 mg/kg/day.

<u>Developmental toxicity:</u> A large number of abortions occurred at the top dose (8 v 0 in the 750 mg/kg/day and control groups, respectively). Mean foetal weight and foetal size (crown-rump length) were slightly, but statistically significantly, lower at the top dose level.

Maternal NOAEL – 250 mg/kg/day
Foetal NOAEL – 250 mg/kg/day</td>
</tr>
<tr>
<td>Developmental Toxicity

OECD 414 (1981)

Rabbit (New Zealand White)

15/group

Ponanna; 1997</td>
<td>Oral (gavage)

Vehicle: aq. Carboxy-methylcellulose

Days 6-18 of gestation</td>
<td>0, 30, 120, 300 or 500 mg/kg/day</td>
<td><u>Maternal toxicity:</u> Treatment related deaths occurred at 120 (2 deaths), 300 (1 death) and at 500 (9 deaths) mg/kg/day. Signs of toxicity (rales, weakness, alopecia, vaginal bleeding) were observed at the top dose level; weakness, nasal discharge, rales and soft stool was also observed at 120 mg/kg/day. Bodyweights were unaffected by treatment.

<u>Developmental toxicity:</u> Because of the large number of maternal deaths at 500 mg/kg/day, very few foetuses were available for examination in this treatment group. A small number of foetuses with variants or malformations were observed at 500 mg/kg/day (see table below), which were considered most likely to be chance findings. At 300 mg/kg/day there was an increased incidence of agenesis of the 4th right lung lobe, a minor morphological variant.
<table>
<tr><th>Parameter / time point</th><th colspan="5">Dose level (mg/kg bw/d)</th></tr>
<tr><th></th><th>0</th><th>30</th><th>120</th><th>300</th><th>500</th></tr>
<tr><th colspan="6">External findings</th></tr>
<tr><td>Litter numbers</td><td>12</td><td>12</td><td>11</td><td>14</td><td>4</td></tr>
<tr><td>No of foetuses</td><td>93</td><td>74</td><td>86</td><td>105</td><td>25</td></tr>
<tr><td>Small foetus (%)</td><td>0</td><td>0</td><td>1.2</td><td>1.0</td><td>4.0 (2)</td></tr>
<tr><td>Forelimbs flexed (%)</td><td>0</td><td>0</td><td>0</td><td>0</td><td>4.0 (2)</td></tr>
<tr><th colspan="6">Visceral findings</th></tr>
<tr><td>Tortuous ureter</td><td>0</td><td>0</td><td>0</td><td>0</td><td>4.0 (1)</td></tr>
<tr><td>Agenesis of 4th right lung lobe</td><td>1.1</td><td>1.4</td><td>2.6</td><td>11.4*</td><td>0</td></tr>
<tr><th colspan="6">Skeletal findings</th></tr>
<tr><td>Arthrogryposis</td><td>0</td><td>0</td><td>0</td><td>0.9</td><td>8.0 (2)*</td></tr>
</table>
*significantly different to controls (p<0.05)[Dunnett’s test] Numbers in brackets indicate total number of foetuses that the finding was observed in

Maternal NOAEL of 30 mg/kg/day
Foetal NOAEL of 120 mg/kg/day</td>
</tr>
</table>

NB: The values for NOAEL and LOAEL are provided for information only: they have already been agreed at a PRAPER expert meeting

The developmental toxicity of metazachlor has been investigated in three developmental studies in rabbit.

No significant maternal toxicity or evidence of treatment related effects were observed in the earliest study. In another study, a large number of abortions were observed at the top dose level.

Severe general maternal toxicity was present at this dose level as two dams died and considerable weight loss was observed, suggesting that the abortions are likely to be a non-specific, stress-related, secondary consequence of maternal toxicity. In a third study, several treatment-related deaths were observed at the top dose indicating that the maximum tolerated dose had been exceeded (9/15 dams died). A few foetuses with abnormalities were observed at this top dose level, but these were considered likely to be chance findings given the small number of affected foetuses. In addition, it is important to note that developmental effects associated with a dose level causing very severe maternal toxicity are of doubtful relevance for classification. The only other noteworthy difference observed in this study was an increase in the incidence of agenesis of the 4th right lung lobe in the mid dose group. A relationship with treatment cannot be excluded, but as agenesis of this particular lung lode is regarded as a minor morphological variation, this isolated finding is of insufficient severity to trigger classification.

Overall, the results of the developmental studies suggest that metazachlor does not cause specific developmental toxicity in rabbits.

5.8.10 Human data

No data

5.8.11 Other relevant information

No data

5.8.12 Summary and discussion of reproductive toxicity

Fertility

Effects on fertility were investigated in a two and a three generation study in rats.

In the two generation study no treatment related effects were observed. In the three generation study, effects were limited to a smaller litter size and effects on offspring (reduced survival index and lower pup weight) at the top dose. These effects were observed at a dose level at which significant maternal toxicity was observed (bodyweight was reduced by 9 % and 12 % at the time of mating in the F1 and F2 generations, respectively). As such it is considered that these effects are likely to be a non-specific secondary consequence of general toxicity and not a direct consequence of administration of metazachlor.

No classification proposed.

Development

Developmental toxicity of metazachlor has been investigated in three studies in mice and three studies in rabbit.

In rats, no malformations of concern were noted and the developmental effects observed were considered to be a secondary non specific consequence of the maternal toxicity and not a direct effect on development.

In rabbits, clear evidence of an adverse effect on pregnancy was limited to high numbers of abortions at the top dose of one study. Severe general maternal toxicity was present at this dose

level as two dams died and significant weight loss was observed, suggesting that the abortions are likely to be a non-specific, stress-related, secondary consequence of maternal toxicity. Overall, there was no evidence of a direct adverse effect on development.

Overall, no classification is proposed.

Directive 67/548/EEC criteria: no classification proposed

Regulation EC/1272/2008: no classification proposed

5.9 Other effects

No relevant data

5.10 Derivation of DNEL(s) or other quantitative or qualitative measure for dose response

Not relevant for this type of dossier.

6 HUMAN HEALTH HAZARD ASSESSMENT OF PHYSICOCHEMICAL PROPERTIES

6.1 Explosivity

Metazachlor was tested in a standard explosivity study (92/69/EEC A14) (De Ryckel (2001) in reference 1). It was found not to be explosive under the influence of a flame and was not sensitive to impact or friction.

No classification for explosivity is proposed.

6.2 Flammability

Metazachlor was tested in a standard flammability study (92/69/EEC A10) (Loffler (1999) in reference 1). The test material did not burn.

Metazachlor was also tested in a standard self ignition temperature study (92/69/EEC A16) (Loffler, (1999) in reference 1). No spontaneous ignition was observed at temperatures up to 400 ^{o}C.

Experience in handling and use indicates that Metazachlor is not pyrophoric and does not react with water to liberate flammable gases.

No classification for flammability is proposed.

6.3 Oxidising potential

Examination of the chemical structure of Metazachlor indicates that it does not contain any chemical groups typical of oxidising agents (De Ryckel (2001) in reference 1**)**.

No classification for oxidising properties is proposed.

7 ENVIRONMENTAL HAZARD ASSESSMENT

A detailed summary of the available studies has been reviewed and their robustness determined under Directive 91/414/EEC and is provided in the Pesticide Assessment Report (DAR) which is attached to the IUCLID 5 dossier. The key information pertinent to determining a classification position is presented below.

The majority of aquatic ecotoxicity testing was undertaken using the active parent substance metazachlor and principle aquatic degradants, namely BH 479-4 and BH 479-6, which were formed up to ~8 % AR in a water/sediment simulation study (Schnoeder, F (2003 – reference 11)[refer to section 4.1.2.3].

Additional aquatic ecotoxicity data are available for metazachlor degradants BH 479-8, BH 479-9, BH 479-11 and BH 479-12. These are primarily soil degradants and were not quantified in aquatic systems. Based on one water/sediment simulation study (Feser-Zugner, W (2000) and (2003) – in reference 11) they are indicated to be present at very low concentrations (~<1 % based on Applied Radioactivity). In addition, the results indicate they are less toxic than the parent metazachlor. On this basis the studies are not included for the purpose of classification and labelling.

Based on metazachlor ecotoxicity testing, algae / higher plant species appear to be more sensitive than fish and invertebrates. This may be the case because metazachlor is a chloracetamide herbicide which stunts and deforms growth via uptake through plant roots and developing shoots. On this basis, testing with aquatic degradants was principally undertaken with algae and higher plant species.

Further aquatic ecotoxicity testing is available with the following Plant Protection Products:
- BAS 479 14 H (content as a.s. Metazachlor: 500 g/l)
- FSG 02094 H (content as a.s. Metazachlor: 507 g/l)

Effects values were considered based on concentrations of product and active substance. These studies are not relevant for the purpose of classification as i) valid test data for the pure substance are available, and ii) the formulation products are mixtures of various components which may affect ecotoxicity.

7.1 Aquatic compartment (including sediment)

7.1.1 Toxicity test results

7.1.1.1 Fish

- Short term toxicity to fish

Metazachlor
Four static GLP 96-hour acute toxicity to fish studies are available following OECD Guideline 203 using metazachlor and three fish species; *Oncorhynchus mykiss* (rainbow trout); *Lepomis macrochirus* (bluegill sunfish); and Cyprinus caprio (common carp).

Study 1 - Oncorhynchus mykiss

Metazachlor (96.6 % purity) was considered stable under the tests conditions with analytical concentrations 80 - 93 % of nominal. Based on the nominal median lethal concentration, the 96-h LC_{50} for *Oncorhynchus mykiss* (Zok, S (2001) – in reference 13) was 8.5 mg a.s./l (95 % C.I. 7.6 – 9.6 mg a.s./l).

Study 2 - Oncorhynchus mykiss
Metazachlor of 97.7 % purity was used. At 0 hours analytical concentrations were 47 – 155 % nominal and some undissolved test substance was observed in exposure solutions. At 72 hours analytical concentrations were 97 – 112 % nominal and no precipitate was observed. Based on nominal concentrations and determined by probit analysis, the study 96-h LC_{50} for *Oncorhynchus mykiss* (Scheerbaum, D (2000) in reference 13) was 8.9 mg/l (95 % C.I. 7.8 – 10 mg/l). Given the analytical measurements, an LC_{50} based on measured data may be more appropriate. However, a revised 96-h LC_{50} is not included here for the purpose of classification and labelling, as it is anticipated that a revised value would not be below the current lowest 7-day E_rC_{50} of 0.0071 mg a.s./l for *Lemna gibba* based on measured data [refer to aquatic plants section]. In addition, the value is similar to the LC_{50} derived from study 1 with the same species.

Study 3 – Lepomis macrochirus
Metazachlor (96.6 % purity) was considered stable under the tests conditions with analytical concentrations within ± 10 % of nominal concentrations. At a nominal concentration of 10 mg/l, 30 % mortality was observed and 100 % mortality observed at 16 mg/l and 25 mg/l. Based on the nominal concentrations, the study 96-h LC_{50} for *Lepomis macrochirus* (Zok, S (2001g) in reference 13) was considered to be about 11 mg a.s./l.

Study 4 – Cyprinus caprio
Metazachlor 97.7 % purity was used. At 0 hours analytical concentrations were 78 – 142 % of nominal concentrations and some undissolved test substance was observed until 24 hours. At 72 hours analytical concentrations were 90 – 107 % nominal and no precipitate was observed. Based on nominal concentrations and determined by probit analysis, the study 96-h LC_{50} for *Cyprinus caprio* (Scheerbaum, D (2000) in reference 13) was 12.3 mg a.s./l (95 % C.I. 7.6 – 9.6 mg a.s./l).

Degradants
One static GLP 96-hour acute toxicity to fish study is available following OECD Guideline 203 using aquatic degradant BH 479-4 (99.3 % purity) and *Oncorhynchus mykiss* (rainbow trout). Analytical measurements were >90 % of nominal concentrations and results were based on nominal concentrations. No adverse effects were observed and the 96-h LC_{50} for *Oncorhynchus mykiss* (Munk, R, Hildebrand, B (1991) in reference 13) was >100 mg/l.

There are no acute fish toxicity data for the aquatic degradant BH 479-6.

- Additional supporting toxicity to fish data

Metazachlor
Two 28-day sub-lethal fish toxicity studies are available following OECD 204 and using *Oncorhynchus mykiss* (rainbow trout).

Study 1
The test used metazachlor (98.7 % purity) and flow-through conditions (Munk, R, Kirsh, P (1990) in reference 13). Analytical measurements were generally >80 % of nominal concentrations but ranged between 74.4 and 111 % of nominal concentrations. Based on nominal concentrations, the NOEC was 2.15 mg a.s./l.

Study 2
The test used metazachlor (97.7 % purity) and semi-static conditions (Scheerbaum, D (2000) in reference 13). Analytical measurements were generally within ± 20 % of nominal concentrations except for the lowest exposure nominal concentration of 0.08 mg/l which was 77% of nominal. Based on nominal concentrations, the NOEC was 2.5 mg/l.

- Long-term toxicity to fish

There are no long-term fish toxicity data.

- Summary

The lowest fish (*Oncorhynchus mykiss*) 96-h LC_{50} was 8.5 mg a.s./l. The study is considered valid and representative of the fish trophic level for the purpose of classification and labelling.

Testing indicates that the aquatic degradant BH 479-4 is less acutely toxic to fish than the parent substance.

7.1.1.2 Aquatic invertebrates

- Short term toxicity to aquatic invertebrates

Metazachlor
Two static GLP 48-hour acute toxicity to *Daphnia magna* (water flea) studies are available following OECD Guideline 202 using metazachlor. Analytical measurements were within ± 20 % of nominal concentrations and results are based on nominal concentrations.

Study 1
Metazachlor (98.7 % purity) was used. The 48-h EC_{50} was 33.7 mg a.s./l (95% C.I. 29.9 – 38.1 mg a.s./l) (Dohmen, G.P (2001) in reference 13).

Study 2
Metazachlor (97.7 % purity) was used. The 48-h EC_{50} was 33 mg/l (Noack, M (2000) in reference 13).

Degradants
Two static GLP 48 hour acute toxicity to Daphnia magna (water flea) following OCED Guideline 202 using aquatic degradants are available.

Study 1
BH 479-4 (99.3 % purity) was used and concentrations were stable within ± 20 % of nominal concentrations (Elendt-Schneider, B (1991) in reference 13). No adverse effects were observed and based on nominal concentrations the 48-h EC_{50} was >100 mg/l.

Study 2
BH 479-6 (98.6 % purity) was used and concentrations were stable within ± 20 % of nominal concentrations (Funk, M (2003) in reference 13). Based on nominal concentrations the 48-h EC_{50} was 84.7 mg/l (95 % C.I. 75.2 – 95.4 mg/l).

- Long term toxicity to aquatic invertebrates

Metazachlor
Two semi-static GLP 21-day sub-lethal toxicity to *Daphnia magna* (water flea) studies using metazachlor are available.

Study 1
The test followed EEC guidelines and used metazachlor (min. 90 % purity) (Jatzek, J.J, Bias, R (1991) in reference 13). Analytical measurements were within ± 20 % of nominal concentrations and results are based on nominal concentrations. Based on reproduction the 21-day NOEC was 6.25 mg a.s./l. While the substance purity was lower than that used in other studies, it is not considered to affect classification and labelling as the result was based on the active substance and is not the lowest NOEC for the species.

Study 2
The test followed OECD guideline 211 and used metazachlor (97.7 % purity) (Noack, M (2000) in reference 13). Analytical measurements were within ± 20 % of nominal concentrations for all but the lowest exposure concentration (nominal 0.1 mg/l) which were 66-82 % nominal. Study results are based on nominal concentrations. Based on reproduction the 21 day NOEC was 0.1 mg/l. If the NOEC was revised to account for measured concentrations, it could be lower. However, this is not presently required for the purpose of classification and labelling of metazachlor.

- Summary

The lowest invertebrate (*Daphnia magna*) 48-h EC_{50} was 33 mg/l. The study is considered valid and representative of the trophic level for the purpose of classification and labelling.

Testing indicates that the aquatic degradants BH 479-4 and BH 479-6 are less acutely toxic to invertebrates than the parent substance.

7.1.1.3 Algae and aquatic plants

Algae

Metazachlor
Four GLP static algal growth inhibition studies are available following OECD Guideline 201 using four different species.

Study 1
The study used metazachlor (98.7 % purity) and *Pseudokirchneriella subcapitata* (green alga formerly *Selenastrum capricornutum*) (Kubitza, J (1998a) in reference 13). Analytical concentrations were within ± 20 % of nominal concentrations and results were based on nominal. The 72-h E_rC_{50} was 0.0318 mg a.s./l (95 % C.I. 0.0243 – 0.0418 mg a.s./l). While a 72-h NOE_rC was not derived, the E_rC_{10} was 0.0061 mg a.s./l.

Study 2
The study used metazachlor (98.7 % purity) and *Anabaena flos-aquae* (blue-green alga) (Kubitza, J (1998b) in reference 13). Analytical concentrations were within ± 20 % of nominal concentrations and results were based on nominal. The 96-h E_rC_{50} reflects the highest exposure concentration and was > 32 mg a.s./l. While a 96-h NOE_rC was not derived, the E_rC_{10} was 13.9 mg a.s./l.

Study 3

The study used metazachlor (97.7 % purity) and *Scenedesmus subspicatus* (green alga) (Scheerbaum, D (2000) in reference 13). The exposure range was 0.00325 to 0.1 mg a.s./l. Analytical concentrations ranged from 21to 102 % of nominal concentrations at 0h and 38 to 84 % of nominal at 72 h. For exposure concentrations of 0.025 mg a.s./l and above, analytical concentrations were greater than 80 % of nominal at study start and end. Results were based on mean measured concentrations. The 72-h E_rC_{50} was 0.031 mg a.s./l (95 % C.I. 0.026 – 0.037 mg a.s./l). The 72-h NOE_rC was 0.0018 mg a.s./l.

Study 4
The study used metazachlor (97.7% purity) and *Navicula pelliculosa* (diatom) (Scheerbaum, C (2000) in reference 13). Analytical concentrations were within ± 20 % of nominal concentrations and results were based on nominal concentrations. The 72-h E_rC_{50} was 72.5 mg a.s./l (95 % C.I. 56.4 – 93.3 mg a.s./l). The 72-h NOE_rC was 3.2 mg a.s./l.

Degradants
Two static GLP 72-hour algal growth inhibition studies following OECD Guideline 201 and using aquatic degradants are available.

Study 1
BH 479-4 (99.3 % purity) was used and concentrations were stable within ± 20 % of nominal concentrations (Dohmen, GP (1993) in reference 13). The study used *Ankistrodesmus bibraianus* (green alga). The study 72-h E_rC_{50} was 9.6 mg/l based on graphical analysis with a 72-h NOE_rC of 1.5 mg/l. Following subsequent statistical analysis, the 72-h E_rC_{50} was revised to 25.7 mg/l (95 % C.I. 24 – 27.6 mg/l).

Study 2
BH 479-4 (94 % purity) was used and concentrations were stable within ± 20 % of nominal concentrations (Scheerbaum, D (2003) in reference 13). The study used *Desmodesmus subspicatus* (green alga). Based on probit analysis, the 72-h E_rC_{50} was 146 mg/l (95 % C.I. 141 – 152 mg/l). The 72-h NOE_rC was 62.5 mg/l.

Aquatic plants

Metazachlor
Three GLP growth inhibition studies are available using *Lemna sp.*.

Study 1
The static study following ASTM guideline E 1415-91 and EPA guidelines used metazachlor (98.7% purity) and *Lemna gibba* (Dohmen, GP (1998) in reference 13). The study was run with and without sediment in test vessels. Analytical concentrations were 95.8 – 131.6 % of nominal concentrations at 0 hours and 87.8 – 111.8 % of nominal concentrations at study completion. Study results were based on nominal concentrations. Without sediment, the 7-d E_rC_{50} was 0.0107 mg a.s./l (95 % C. I. 0.0091 – 0.0123 mg a.s./l). The 7-d NOE_rC was 0.0006 mg a.s./l. With sediment, the 7-d E_rC_{50} was 0.0208 mg a.s./l (95 % C.I. 0.015 – 0.0289 mg a.s./l). The 7-d NOE_rC was 0.0016 mg a.s./l.

Study 2
The static study following ASTM guideline E 1415-91 and EPA guidelines used metazachlor (98.7% purity) and *Lemna gibba* (Junker, M, Kubitza, J (2003) in reference 13). The study examined effects and recovery. The 72-h NOEC was 0.003 mg/l. No EC_{50} was determined.

Study 3

The semi-static study following ASTM guideline E 1415-91 and EPA guidelines used metazachlor (97.7% purity) and *Lemna gibba* (Scheerbaum, D (2000) in reference 13). Results are based on mean measured concentrations. The 7-d E_rC_{50} was 0.0071 mg a.s./l (95 % C.I. 0.0013 – 0.038 mg a.s./l). The 7-d NOE_rC was 0.000193 mg a.s./l. The 14-d E_rC_{50} was 0.0065 mg a.s./l (95 % C.I. 0.0035 – 0.012 mg a.s./l). The 14-d NOE_rC was 0.000193 mg a.s./l.

Degradants
Two GLP 7 day growth inhibition studies following OCED draft Guideline 221, and *Lemna spp*. are available for the following aquatic degradants.

Study 1
BH 479-4 (94 % purity) was used with *Lemna minor* under semi-static conditions (Scheerbaum, d (2003) in reference 13). Concentrations were stable within ± 20 % of nominal concentrations. The 7-d E_rC_{50} was > 100 mg/l and the 7-d NOE_rC was > 100 mg/l.

Study 2
BH 479-6 (98.6 % purity) was used with *Lemna gibba* under semi-static conditions (Junker, M (2003) in reference 13). Concentrations were stable within ± 20 % of nominal concentrations. The 7-d E_rC_{50} was > 100 mg/l and the 7-d E_rC_{10} was 2.53 mg/l.

- Summary

The lowest algal and aquatic plants effects value is a 7-d E_rC_{50} 0.0071 mg a.s./l for *L. gibba* based on mean measured concentrations. The study is considered valid and representative of the trophic level for the purpose of classification and labelling.

Testing with aquatic degradants BH 479-4 and BH 479-6 indicates these degradants are less toxic to algae and aquatic plants than the parent substance.

7.1.1.4 Sediment organisms

One 28 day study using metazachlor (97.7 % purity) and *Chironomus riparius* (2-3 days old) following BBA guideline proposal (1995) is available as supporting information (Scheerbaum, D (2000) in reference 13). Metazachlor was added to the aqueous phase of a water-sediment system. It was observed to dissipate from water to sediment and degrade in the system with 13 - 42 % recovery in water and 7 - 27 % recovery in sediment by day 28. Results were based on nominal concentrations. Using probit analysis, the EC_{50} was 17.6 mg a.s./l (95 % C.I. 16.8 – 18.5 mg a.s./l). The NOEC was 9.8 mg a.s./l based on emergence. Expressed as a sediment concentration, the NOEC is 7.93 mg a.s./kg.

7.1.1.5 Other aquatic organisms

No additional data available.

7.1.2 Calculation of Predicted No Effect Concentration (PNEC)

Not relevant to this type of dossier.

7.2 Terrestrial compartment

Not relevant to this type of dossier.

7.3 Atmospheric compartment

Not relevant to this type of dossier.

7.4 Microbiological activity in sewage treatment systems

Not relevant to this type of dossier.

7.5 Calculation of Predicted No Effect Concentration for secondary poisoning (PNEC oral)

Not relevant to this type of dossier.

7.6 Conclusion on the environmental classification and labelling

Hydrolysis and aquatic photolysis are not considered significant removal pathways for metazachlor.

On the basis of a ready biodegradation study, metazachlor is not considered readily biodegradable.

The fate of metazachlor in four aerobic water/sediment systems indicates that mineralisation to carbon dioxide is slow. The relatively short dissipation half-life in water and low degradant concentrations in water indicates that metazachlor is partitioned to the sediment phase where degradation and formation of non-extractable sediment residues occurs. The estimated DT_{50} values indicates that metazachlor does not undergo significant mineralisation with less than 70% over 28 days. This means that metazachlor is considered not readily biodegradable for the purpose of classification and labelling.

Based on the low measured log K_{ow} values (2.49 and 2.5) and the estimated BCF_{fish} (26.6 $l/kg_{wet\ fish}$), metazachlor is considered to have a low bioaccumulation potential.

Metazachlor and its degradants exhibited limited acute toxicity to fish and invertebrates compared to other trophic levels, with the lowest 48-h LC_{50} of 8.5 mg a.s. /l for fish. It is acutely toxic to some algae species with a lowest 72-h E_rC_{50} of 0.031 mg/l for *Scenedesmus subspicatus*. Metazachlor is also acutely toxic to the aquatic plant *Lemna spp*. with the lowest 7-d E_rC_{50} 0.0071 mg a.s./l based on mean measured concentrations. The study was performed according to GLP and standard test guidelines and all validation criteria were met. *Lemna spp.* are considered a representative aquatic species for the primary producer trophic level and appear to be more sensitive than algae, fish and invertebrates. In summary, the study is acceptable for the purpose of classification under Directive 67/548/EEC and the CLP Regulation since a suitable test method and representative aquatic species were used.

Following Directive 67/548/EEC, metazachlor should be classified Dangerous for the Environment with the following risk and safety phrases:
N Dangerous for the Environment
R50 Very toxic to aquatic organisms
R53 May cause long term effects in the environment
S60 This material and its container must be disposed of as hazardous waste
S61 Avoid release to the environment. Refer to special instructions/Safety Data Sheet

The following Special Concentration Limits should apply:

Classification of the preparation		
N, R50-53	N, R51-53	R52-53
Cn ≥ 0.25 %	0.025 % ≤ Cn <0.25 %	0.0025 % ≤ Cn <0.025 %

Where Cn is the concentration of metazachlor in the preparation.

Based on the CLP Regulation, metazachlor should be classified:
Aquatic Acute 1, Aquatic Chronic 1
H400 'Very toxic to aquatic life', H410 'Very toxic to aquatic life with long lasting effects'
Signal Word: 'Warning' and environmental warning label.
An M factor of 100 is applicable based on 0.001 <L(E)C_{50} ≤0.01 mg/l.

JUSTIFICATION THAT ACTION IS REQUIRED ON A COMMUNITY-WIDE BASIS

Metazachlor is a chloroacetanilide herbicide used on oil seed rape. In 2008 it was approved for Annex I listing as a 3A review compound under Council Directive 91/414/EEC, with the UK as rapporteur Member State. In accordance with Article 36(2) of the CLP Regulation, Metazachlor should now be considered for harmonised classification and labelling. Therefore, this proposal considers all human health and environmental endpoints.

OTHER INFORMATION

This substance has been reviewed under Council Directive 91/414/EEC, with the rapporteur Member State being the United Kingdom. The studies evaluated in this dossier were taken from the pesticide assessment report; where necessary, the full study reports were consulted, but these are generally not publically available. Where other information from additional references has been sources, this is indicated.

References

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2. Pesticide Assessment Report (DAR) – public Version – initial risk assessment provided by the rapporteur Member State United Kingdom for the existing active substance metazachlor of the third stage (part A) of the review programme referred to in Article 8 (2) of the Council Directive 91/414/EEC. Volume 3, Annex B.6, 2005
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3. Buesen, R, Strauss V, Gröters, S, Fabian E, van Ravenzwaay B (2009a) BAS 479 H (Metazachlor). Microcrystallisation in the urinary bladder and enzyme induction in the liver and kidney of Wistar rats; Administration in the diet over 2 weeks (BASF Doc ID: 2009/1048393). *Unpublished*.

4. Buesen, R, Strauss V, Gröters, S, van Ravenzwaay B (2009b) BAS 479 H (Metazachlor). Thyroid Hormone Study. Repeated Dose Oral Toxicity Study in Wistar Rats; Administration in the Diet for 4 weeks (BASF Doc ID: 2009/1048392). *Unpublished.*

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7. Buesen R, Strauss V, Gröters, S, Fabian E, van Ravenzwaay B (2009d) BAS 479 H (Metazachlor). Microcrystallisation in the urinary bladder and enzyme induction in the liver and kidney of CD-1 mice; Administration in the diet over 2 weeks (BASF Doc ID: 2009/1048394). *Unpublished*

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stage (part A) of the review programme referred to in Article 8 (2) of the Council Directive 91/414/EEC. Volume 3, Annex B.8, 2005
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13. European Chemicals Bureau Joint Research Centre (2003) Technical Guidance Document on Risk Assessment.

14. Pesticide Assessment Report (DAR) – public Version – initial risk assessment provided by the rapporteur Member State United Kingdom for the existing active substance metazachlor of the third stage (part A) of the review programme referred to in Article 8 (2) of the Council Directive 91/414/EEC. Volume 3, Annex B.9, 2005
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http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902028580.htm

Appendix 1

Carcinogenicity studies

Table 1. Rat data re-examined

The tables show the incidences observed in the original study (original) and the incidences observed following re-examination by the BASF pathologists (internal) and the PWG (PWG).

Dose schedule [reference]	Dose levels	Observations and remarks (effects of major toxicological significance)

<table>
<tr>
<td>Daily in diet

Rat: Wistar

OECD 453

50/sex/group exposed for 2 years
10/ sex of controls and 20/sex of 8000 ppm exposed for 1 year

Re-analysis of the results of the Krishnappa, 2002, see reference 1 of this Annex</td>
<td>0, 200, 2000 and 8000 ppm

Corresponds to 0, 9, 87 and 361 mg/kg/day in males 0, 12, 114 and 442 mg/kg/day in females</td>
<td>Liver tumours

<table>
<tr><td>Review</td><td colspan="4">Females</td></tr>
<tr><td>Dose (ppm)</td><td>0</td><td>200</td><td>2000</td><td>8000</td></tr>
<tr><td colspan="5">Hepatocellular adenoma</td></tr>
<tr><td>Original</td><td>1 (2 %)</td><td>0</td><td>1 (2 %)</td><td>8(16%)</td></tr>
<tr><td>Internal</td><td>1 (2 %)</td><td>0</td><td>1 (2 %)</td><td>6 (12%)</td></tr>
<tr><td>PWG</td><td>1 (2 %)</td><td>0</td><td>0</td><td>6 (12%)</td></tr>
<tr><td colspan="5">Historical control 1.13 % (0-6%) Dates: 08/96-09/05</td></tr>
<tr><td colspan="5">Hepatocellular carcinoma</td></tr>
<tr><td>Original</td><td>0</td><td>0</td><td>2 (4 %)</td><td>1 (2 %)</td></tr>
<tr><td>Internal</td><td>0</td><td>0</td><td>2 (4 %)</td><td>2 (4 %)</td></tr>
<tr><td>PWG</td><td>0</td><td>0</td><td>2 (4 %)</td><td>2 (4 %)</td></tr>
<tr><td colspan="5">Historical control 0 % Dates: 08/96-09/05</td></tr>
<tr><td colspan="5">Combined (adenoma/ carcinoma)</td></tr>
<tr><td>Original</td><td>1 (2 %)</td><td>0</td><td>3 (6 %)</td><td>9 (18%)</td></tr>
<tr><td>Internal</td><td>1 (2 %)</td><td>0</td><td>3 (6 %)</td><td>8 (16%)</td></tr>
<tr><td>PWG</td><td>1 (2 %)</td><td>0</td><td>2 (4 %)</td><td>8 (16%)</td></tr>
</table>
</td>
</tr>
<tr>
<td>Daily in diet

Rat: Sprague-Dawley

Non-guideline

2-years

50/sex/group exposed for 2 years
10/sex/group exposed for 1 year
15/sex/grp as satellite for blood sampling exposed for 2 years

Re-analysis of the results of Hunter B et al, 1983a, and Hunter B et al, 1983b, see reference 1 of this Annex for liver and thyroid reanalysis and reference 2 of this Annex for Leydig cell reanalysis</td>
<td>0, 100, 500, 2000 and 6000 ppm.

Corresponds to 0, 3.2, 18, 73 and 226 mg/kg/day in males 0, 4, 21, 88, 272 mg/kg/day in females.</td>
<td>The values presented in normal typeface are the incidences observed in Hunter B et al, 1983a. The values presented in italics are the incidences observed in Hunter B et al, 1983b

Liver tumours

<table>
<tr><td></td><td colspan="6">Males</td></tr>
<tr><td>Dose (ppm)</td><td>0</td><td>100</td><td>500</td><td>2000</td><td>6000</td></tr>
<tr><td colspan="6">Hepatocellular adenoma</td></tr>
<tr><td>Original</td><td>0</td><td>2 (4 %)</td><td>0</td><td>0</td><td>0</td><td>2 (4 %)</td></tr>
<tr><td>Internal</td><td>1 (2 %)</td><td>1 (2 %)</td><td>0</td><td>0</td><td>0</td><td>2 (4 %)</td></tr>
<tr><td>PWG</td><td>2 (4 %)</td><td>1 (2 %)</td><td>0</td><td>0</td><td>0</td><td>2 (4 %)</td></tr>
<tr><td colspan="6">Historical control: 1.13% (0-4%) Date: 03/78-10/84</td></tr>
<tr><td colspan="6">Hepatocellular carcinoma</td></tr>
<tr><td>Original</td><td>2 (4 %)</td><td>1(2 %)</td><td>1(2 %)</td><td>1 (2 %)</td><td>2 (4 %)</td><td>2 (4 %)</td></tr>
<tr><td>Internal</td><td>2 (4 %)</td><td>2 (4 %)</td><td>1 (2 %)</td><td>1 (2 %)</td><td>2 (4 %)</td><td>2 (4 %)</td></tr>
<tr><td>PWG</td><td>0</td><td>1(2 %)</td><td>1(2 %)</td><td>1 (2 %)</td><td>2 (4 %)</td><td>2 (4 %)</td></tr>
<tr><td colspan="6">Historical control: 1.97% (0-6%) Date: 03/78-10/84</td></tr>
<tr><td colspan="6">Combined (adenoma/ carcinoma)</td></tr>
<tr><td>Original</td><td>2 (4 %)</td><td>3 (6 %)</td><td>1 (2 %)</td><td>1 (2 %)</td><td>2 (4 %)</td><td>4 (8 %)</td></tr>
<tr><td>Internal</td><td>3 (6 %)</td><td>3 (6 %)</td><td>1 (2 %)</td><td>1 (2 %)</td><td>2 (4 %)</td><td>4 (8 %)</td></tr>
<tr><td>PWG</td><td>2 (4 %)</td><td>2 (4 %)</td><td>1 (2 %)</td><td>1 (2 %)</td><td>2 (4 %)</td><td>4 (8 %)</td></tr>
</table>

Thyroid
Parafollicular tumours

<table>
<tr><td></td><td colspan="6">Males</td></tr>
<tr><td>Dose (ppm)</td><td>0</td><td>0</td><td>100</td><td>500</td><td>2000</td><td>6000</td></tr>
<tr><td colspan="6">Parafollicular hyperplasia</td></tr>
<tr><td>Original</td><td>0</td><td>3(6 %)</td><td>1(2 %)</td><td>3 (6 %)</td><td>2 (4 %)</td><td>1 (2 %)</td></tr>
<tr><td>Internal</td><td>3 (6 %)</td><td>6 (12%)</td><td>0</td><td>9 (18%)</td><td>6 (12%)</td><td>1 (2 %)</td></tr>
<tr><td>PWG</td><td>3 (6 %)</td><td>3 (6 %)</td><td>0</td><td>6 (12%)</td><td>3 (6 %)</td><td>0</td></tr>
<tr><td colspan="6">Parafollicular cells (ie C-cell) adenoma</td></tr>
<tr><td>Original</td><td>2 (4 %)</td><td>1 (2 %)</td><td>2 (4 %)</td><td>1(2%)</td><td>5 (10%)</td><td>5 (10%)</td></tr>
</table>
</td>
</tr>
</table>

Internal	5(10 %)	*8 (16%)*	*2 (4 %)*	4(8 %)	7(14 %)	7(14 %)
PWG	5(10 %)	*8 (16%)*	*2 (4 %)*	6(12 %)	9(18 %)	6(12 %)
Historical control 0.63 % (0-4 %) Date: 03/78-10/84						
C-cell carcinoma						
Original	0	*8 (16%)*	*1 (2 %)*	1 (2 %)	2 (4 %)	3 (6 %)
Internal	0	*2 (4 %)*	*1 (2 %)*	2 (4 %)	0	1 (2 %)
PWG	0	*2 (4 %)*	*1 (2 %)*	2 (4 %)	0	3 (6 %)
Historical control 6.93% (0-18%) Date: 03/78-10/84						
Combined (adenoma/ carcinoma)						
Original	2 (4 %)	*9 (18%)*	*3 (6 %)*	2 (4 %)	7 (14%)	8 (16%)
Internal	5(10 %)	*10(20%)*	*3 (6%)*	6(12 %)	7(14 %)	8(16 %)
PWG	5(10 %)	*10(20%)*	*3 (6%)*	8 (16%)	9(18 %)	9(18 %)

Thyroid
Follicular tumours

	Males					
Dose (ppm)	0	*0*	*100*	500	2000	6000
Follicular cell hyperplasia						
Original	0	*0*	*0*	0	0	0
Internal	1 (2 %)	*2 (4 %)*	*3 (6 %)*	2 (4 %)	3 (6 %)	8 (16%)
PWG	1 (2 %)	*2 (4 %)*	*3 (6 %)*	2 (4 %)	3 (6 %)	6 (12%)
Follicular cell adenoma						
Original	0	*1(2 %)*	*2 (4%)**	1(2 %)	2(4 %)	3(6 %)**
Internal	0	*1 (2 %)*	*1 (2 %)*	1(2 %)	2(4 %)	3(6 %)
PWG	0	*2 (4 %)*	*1 (2 %)*	1(2 %)	1(2 %)	5(10 %)
Historical control: 4.7 % (0-13%) Date: 03/78-10/84						
Follicular cell carcinoma						
Original	0	*2 (4 %)*	*0*	0	0	1(2 %)
Internal	0	*1 (2 %)*	*0*	0	0	1(2 %)
PWG	0	*0*	*0*	0	1(2 %)	1(2 %)
Historical control: 1.18 % (0-8%) Date: 03/78-10/84						
Combined (adenoma/ carcinoma)						
Original	0	*3 (6 %)*	*2 (4 %)*	1(2 %)	2(4 %)	4(8%)
Internal	0	*2 (4 %)*	*1 (2 %)*	1(2 %)	2(4 %)	4(8%)
PWG	0	*2 (4 %)*	*1 (2 %)*	1(2 %)	2 (4 %)	6(12 %)

* - Personal communication from industry, this value should be 3 not 4 as reported in the PWG report.
**- a value of 3 is given in the PWG report. However, in the original study report an incidence of 4 is reported.

Leydig cells

	Males			
Dose (ppm)	0	500	2000	6000
Leydig cell hyperplasia (focal)				
Original	0	1(1.7%)	0	0
Internal	8 (13%)	6 (10%)	3 (5 %)	8(13%)
PWG	8(13%)	6(10%)	3(5%)	9(15 %)
No historical control data				
Leydig cell adenoma				
Original	1(1.7%)	1(1.7%)	1(1.7%)	4(6.7%)
Internal	2(3.3%)	2(3.3%)	1(1.7%)	4(6.7%)
PWG	2(3.3%)	2(3.3%)	1(1.7%)	4(6.7%)
Historical control: 5.7% (0-16 %) Date: 09/83-10/02				

Table 2 mice data re-examined

The tables show the incidences observed in the original study (original) and the incidences observed following re-examination by the BASF pathologists (internal) and the PWG (PWG). The incidence of bladder tumours was also re-examined by another external reviewer (external). Please note that the findings for the lymphoreticular system have had to be presented separately as the types of findings were reclassified.

<table>
<tr><th>Dose schedule [reference]</th><th>Dose levels</th><th>Observations and remarks (effects of major toxicological significance)</th></tr>
<tr><td>Daily in the diet

Swiss

OECD 453

18 month

50/sex/group

Reanalysis of the results of Kumar, 2003, see reference 3 of this Annex</td><td>0, 100, 1000 or 4000 ppm

Corresponds to 15, 154 and 578 mg/kg/day in males
0, 16, 163 and 640 mg/kg/day in females</td><td>Bladder tumours
<table>
<tr><td></td><td colspan="4">males</td><td colspan="4">Females</td></tr>
<tr><td>Dose (ppm)</td><td>0</td><td>100</td><td>1000</td><td>4000</td><td>0</td><td>100</td><td>1000</td><td>4000</td></tr>
<tr><td colspan="5">Epithelia/ urothelial hyperplasia (diffuse)</td><td colspan="4"></td></tr>
<tr><td>Original</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>External</td><td>5 (10%)</td><td>1(6%)</td><td>2 (10%)</td><td>27 (54%)</td><td>2 (4%)</td><td>1 (6%)</td><td>5 (36%)</td><td>36 (72%)</td></tr>
<tr><td>Internal</td><td>0</td><td>0</td><td>0</td><td>37(74%)</td><td>0</td><td>0</td><td>0</td><td>24(48%)</td></tr>
<tr><td>PWG</td><td>4(8%)</td><td>1(2%)</td><td>2(4%)</td><td>38(76%)</td><td>4(8%)</td><td>1(2%)</td><td>4(8%)</td><td>39(78%)</td></tr>
<tr><td colspan="5">Epithelial/ urothelial hyperplasia (focal)</td><td colspan="4"></td></tr>
<tr><td>Original</td><td>1 (2%)</td><td>0</td><td>0</td><td>1 (2 %)</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>External</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>Internal</td><td>1(2%)</td><td>0</td><td>2(4%)</td><td>1 (2 %)</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>PWG</td><td>0</td><td>0</td><td>2(4%)</td><td>1 (2 %)</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td colspan="5">Transitional cell papilloma</td><td colspan="4"></td></tr>
<tr><td>Original</td><td>0</td><td>0</td><td>1(5%)</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>External</td><td>1(2%)</td><td>0</td><td>1(5%)</td><td>1(2%)</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>Internal</td><td>1(2%)</td><td>0</td><td>1(2%)</td><td>1 (2 %)</td><td>0</td><td>1(2%)</td><td>0</td><td>2 (4 %)</td></tr>
<tr><td>PWG</td><td>1(2%)</td><td>0</td><td>1(2%)</td><td>1(2 %)</td><td>0</td><td>1(2%)</td><td>0</td><td>2 (4 %)</td></tr>
<tr><td colspan="5">Transitional cell carcinoma</td><td colspan="4"></td></tr>
<tr><td>Original</td><td>0</td><td>0</td><td>0</td><td>1 (2 %)</td><td>0</td><td>0</td><td>0</td><td>2 (4 %)</td></tr>
<tr><td>External</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>2 (4 %)</td></tr>
<tr><td>Internal</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>PWG</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
</table></td></tr>
<tr><td>Daily in the diet

CD-1

US-EPA

2-year

50/sex/group exposed for 2 years
10/sex/group exposed for 1 year

Reanalysis of the results of Barnard et al, 1983, see</td><td>0, 200, 700 or 2500 ppm

Corresponds to 19, 72, and 252 mg/kg/day in males
0, 21, 74 and 273 mg/kg/day in females</td><td>It is unclear whether the historical control data was derived from 18 month or 2 year studies.

Liver
<table>
<tr><td></td><td colspan="4">Females</td></tr>
<tr><td>Dose (ppm)</td><td>0</td><td>200</td><td>700</td><td>2500</td></tr>
<tr><td colspan="5">Hepatocellular adenoma</td></tr>
<tr><td>Original</td><td>0</td><td>0</td><td>1 (2%)</td><td>3 (6%)</td></tr>
<tr><td>Internal</td><td>0</td><td>0</td><td>1 (2%)</td><td>3(6 %)*</td></tr>
<tr><td>PWG</td><td>1 (2 %)</td><td>0</td><td>1 (2%)</td><td>4 (8 %)</td></tr>
<tr><td colspan="5">Historical control (06/78 – 10/84) 3.49 % (0-9.8%)</td></tr>
<tr><td colspan="5">Hepatocellular carcinoma</td></tr>
<tr><td>Original</td><td>0</td><td>1 (2 %)</td><td>0</td><td>1 (2 %)</td></tr>
<tr><td>Internal</td><td>0</td><td>1 (2 %)</td><td>0</td><td>1(2 %)**</td></tr>
<tr><td>PWG</td><td>0</td><td>1 (2 %)</td><td>0</td><td>0</td></tr>
<tr><td colspan="5">Historical control (06/78-10/84) 1.14 (0-4 %)</td></tr>
<tr><td colspan="5">Combined adenoma/ carcinoma</td></tr>
<tr><td>Original</td><td>0</td><td>1 (2 %)</td><td>1 (2 %)</td><td>4 (8 %)</td></tr>
</table></td></tr>
</table>

reference of 4 of this Annex for liver tumours, reference 5 for kidney tumours and reference 6 for reanalysis of the lymphoreticular findings.

Internal	0	1 (2 %)	1 (2 %)	4 (8 %)
PWG	1 (2 %)	1 (2 %)	1 (2 %)	4 (8 %)

*-Personal communication from industry, this incidence should be 3, not 4 as reported in the PWG report.
**- Personal communication from industry, this incidence should be 1, not 0 as reported in the PWG report.

Kidney

	males			
Dose (ppm)	0	200	700	2500
Cortical (renal tubule) adenoma/ papillary cystadenoma				
Original	0	1 (2%)	3 (6%)	4 (8%)*
Internal	0	1 (2%)	3 (6%)	4 (8%)
PWG	0	1 (2%)	4 (8%)	4 (8%)
Historical control (tubule) 0.3 % (0-2 %) (Dates: 06/78-10/84)				
Cortical (renal tubule) carcinoma				
Original	0	0	1 (2%)	0
Internal	0	0	1 (2%)	0
PWG	0	0	0	0
Historical control (tubule) 0.27 % (0-3.9 %) (Dates:06/78-10/84)				
Combined adenoma/ carcinoma				
Original	0	1 (2%)	4 (8%)	4 (8%)
Internal	0	1 (2%)	4 (8%)	4 (8%)
PWG	0	1 (2%)	4 (8%)	4 (8%)

* This value is presented as 3 (6 %) in the pesticide assessment review.

Lymphoreticular system

Original study findings

	males			
Dose (ppm)	0	200	700	2500
Lymphoblastic leukaemia				
	0	0	0	2 (4%)
Lymphosarcoma				
	3 (6%)	4 (13%)	0	4 (8%)
Reticulum cell sarcoma				
	1(2%)	1(2%)	4 (13%)	0
Lymphoid leukaemia				
	1(2%)	0	0	0
Myeloid Leukaemia				
	0	1(3%)	1(3%)	0
Combined “lymphoreticular tumours”				
	5(10%)	6(20%)	5(17%)	6(12%)

Internal review findings

	males			
Dose (ppm)	0	200	700	2500
Lymphoma Malignant				
	5(10%)	6(20%)	3(10%)	6(12%)
Lymphocytic type				
	0	2(7%)	0	1(2%)
Lymphoblastic type				
	0	2(7%)	0	0
Pleomorphic type				
	3(6%)	2(7%)	3(10%)	4(8%)
NOS				
	2(4%)	0	0	1(2%)
Histiocytic sarcoma				
	1(2%)	0	2(7%)	0

		Combined 'lymphoreticular tumours'				
			6(12%)	6(20%)	5(17%)	6(12%)
		PWG review				
			males			
		Dose (ppm)	0	200	700	2500
		Lymphoma malignant				
			5(10%)	6(20%)	3(10%)	6(12%)
		Histiocytic sarcoma				
			1(2%)	0	2(7%)	0
		Combined "lymporeticular tumours"				
			6(12%)	6(20%)	5(17%)	6(12%)

NB. Not all animals were evaluated in each dose group; this is reflected by some of the percentage incidences presented.

References

1. Wall H (2008) Pathology Working Group (PWG) review of the carcinogenic potential of metazachlor: Liver and Thyroid gland of Sprague-Dawley and Wistar rats. *Unpublished*

2. Wall H (2008) Pathology Working Group (PWG) review of the carcinogenic potential of metazachlor: Interstitial cell (Leydig) cell tumours of Sprague-Dawley rats. *Unpublished*

3. Wall H (2008) Pathology Working Group (PWG) review of the carcinogenic potential of metazachlor: Proliferative lesions in the urinary bladder in Swiss Albino Mice. *Unpublished*

4. Wall H (2008) Pathology Working Group (PWG) review of the carcinogenic potential of metazachlor: Liver tumours of CD-1 (Charles River) female mice. *Unpublished*

5. Wall H (2008) Pathology Working Group (PWG) review of the carcinogenic potential of metazachlor: Kidney tumours in male mice. *Unpublished*

6. Wall H (2008) Pathology Working Group (PWG) review of the carcinogenic potential of metazachlor: lymphoreticular tumours in male CD-1 (Charles River) mice. *Unpublished*