Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling at Community level of

metazachlor

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

Adopted

8 March 2011

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CLH-O-0000001586-69-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT COMMUNITY LEVEL

In accordance with Article 37 (4) of the Regulation (EC) No 1272/2008 (CLP Regulation), the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling of

Substance Name: ***Metazachlor***

EC Number: 266-583-0

CAS Number: 67129-08-2

The proposal was submitted by ***United Kingdom*** and received by RAC on ***30 November 2009***

The proposed harmonised classification

	CLP Regulation (EC) No 1272/2008	Directive 67/548/EEC (criteria)
Current entry in Annex VI CLP Regulation	-	-
Proposal by dossier submitter for consideration by RAC	Skin Sens. 1; H317 Carc. 2; H351 Aquatic Acute 1; H400 Aquatic Chronic 1; H410	R43 Carc. Cat. 3; R40 N; R50/53
Resulting harmonised classification, proposed future entry in Annex VI CLP Regulation.	Skin Sens. 1; H317 Carc. 2; H351 Aquatic Acute 1; H400 Aquatic Chronic 1; H410 M-factor =100	R43 Carc. Cat. 3; R40 N; R50/53 Specific concentration limits: N; R50/53: C ≥ 0,25 % N; R51/53: 0,025 % ≤ C < 0,25 % R52/53: 0,0025 % ≤ C < 0,025 %

PROCESS FOR ADOPTION OF THE OPINION

United Kingdom has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was

made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/consultations/harmonised_cl_en.asp*** on 12 March 2010.Parties concerned and MSCAs were invited to submit comments and contributions by ***26 April 2010.***

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: ***Agnes Schulte***
Co-rapporteur, appointed by RAC: ***Katalin Gruiz***

The RAC opinion on the proposed harmonised classification and labelling has been reached on ***8th March 2011,*** in accordance with Article 37 (4) of the CLP Regulation, giving parties concerned the opportunity to comment. ***Comments received are compiled in Annex 2.***

The RAC Opinion was adopted by ***consensus.***

OPINION OF RAC

RAC adopted the opinion that ***metazachlor*** should be classified and labelled as follows:

Classification & Labelling in accordance with the CLP Regulation:

Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
				Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
	Metazachlor	266-583-0	67129-08-2	Skin Sens. 1* Carc. 2 Aquatic Acute 1 Aquatic Chronic 1	H317 H351 H400 H410	GHS07 GHS08 GHS09 Wng	H317 H351 H410		M=100**	

* The proposed Classification for Skin Sensitisation according to the criteria in the 2nd ATP to the CLP Regulation should be Skin Sens. 1B

** The proposed M-factors according to the criteria in the 2nd ATP to the CLP Regulation should be Acute=100 and Chronic=100

Classification & labelling in accordance with Directive 67/548/EEC:

Index No	International Chemical Identification	EC No	CAS No	Classification	Labelling	Concentration Limits	Notes
	Metazachlor	266-583-0	67129-08-2	R43 Carc. Cat. 3; R40 N; R50/53	Xn; N R: 40-43-50/53 S: (2-)36-37-60-61	N; R50/53: C ≥ 0,25 % N; R51/53: 0,025 % ≤ C < 0,25 % R52/53: 0,0025 % ≤ C < 0,025 %	

SCIENTIFIC GROUNDS FOR THE OPINION

The opinion relates to those hazard classes that have been reviewed in the proposal for harmonised classification and labelling on metazachlor as submitted by United Kingdom.

The opinion takes into account the comments of MSCAs and parties concerned provided in accordance with Art. 37(4) of the CLP Regulation.

The Background Document (Annex 1) gives the detailed scientific grounds for the Opinion.

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, the proposal on metazachlor considers all human health and environmental endpoints for harmonised classification and labelling (see Background Document, Annex 1).

This Opinion proposes harmonised classification and labelling to the endpoints of carcinogenicity, skin sensitization, acute aquatic toxicity and chronic aquatic toxicity.

Acute toxicity

No classification is proposed for this endpoint under either Directive 67/548/EEC or CLP Regulation.

Specific target organ toxicity – single exposure

There was no human data and no evidence of any specific, non lethal target organ toxicity arising from a single exposure to metazachlor that require classification as STOT-SE under the CLP Regulation.

Irritation

Data do not support classification for skin or eye irritation under either Directive 67/548/EEC or CLP Regulation. No classification is proposed for respiratory tract irritation.

Skin 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 responding at >1% intradermal induction dose), classification as skin sensitisation category 1B (H317) under the new criteria of CLP regulation (2nd ATP) and as Xi; R 43 under Directive 67/548/EEC are proposed.

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

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 according to the DSD 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 show 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 DSD.

Under the CLP Regulation, the classification cut-off values (guidance values) for STOT-RE are higher than in DSD: 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.

No treatment-related effects were observed in a dermal 28-day toxicity study in rats. No classification is warranted for this route. No data are available for the inhalation route.

Mutagenicity

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

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).

Liver

Considerations supporting the conclusion that liver tumours in rat are related to the exposure of metazachlor are:

- The study of Krishnappa (2002) was identified as key study on liver carcinogenicity of metazachlor. Metazachlor induced liver tumours in female Wistar rats. Incidences of liver adenomas were observed in female rats receiving 0, 200, 2000 or 8000 ppm at incidences of 2%, 0%, 2% and 16%, liver cell carcinomas occurred at 0%, 0%, 4% and 2% incidence rates.

- No clear liver tumour response was seen in male Wistar rats. The Krishnappa study revealed one liver adenoma (out of 50 mid and high dose males) compared to none in the male control group. However the low incidence of liver adenomas in male rats is not clearly attributable to a sex-specific response. The facts that increased incidences of liver lesions including liver foci/masses and hepatocellular hypertrophy and increased liver weight were similarly observed in females and males and that male rats received lower doses on the basis of mg/kg bodyweight per day dosage (361 mg/kg in high dose males versus 442 mg/kg in females) argue against an interpretation that liver tumours were clearly limited to the female sex.

- The study is valid and all dose levels tested were reliable for evaluating chronic toxicity and carcinogenicity of metazachlor. High doses administered in this Wistar rat study were well tolerated and thus were below the level of MTD. Up to 8000 ppm (361/442 mg/kg/day in male/female rats) survival in the carcinogenicity study of Krishnappa (2002) was unaffected and no signs of toxicity were observed. Lower body weight (-11/10%) were concordant to lower food consumption (-8/13% for high dose males/females). This observation is in line with data from 28-day and 90-day studies, which demonstrated that Wistar rats tolerated doses up 15000 ppm without any sign of clinical toxicity.

- Increases in liver weight, increased gamma-glutamyl-transferase activity, increased plasma total bilirubin levels were identified as dose-related specific effects rather than as effects of nonspecific (MTD-relevant) toxicity.

- Low incidences of liver tumours in control groups (0 and 1 adenoma/50 male and females, respectively) (and also absence of liver tumours in low dose groups) confirm that uncertainties due to (high) spontaneous incidences of the strain or species used are not present. Tumour incidences on internal controls are generally of higher significance than historical control data unless there is an argument that internal controls are invalid.

- If historical control data were taken into account, incidences of liver adenoma and carcinoma were above mean values of in-house historical control data and above mean values of internal historical controls and mean values of the RITA database on historical control for Wistar rats. Incidences were at the size of maximum values or below the upper range of tumours commonly seen in this strain in the RITA database (see Table below).

- Liver carcinomas observed in mid and high dose females do support metazaclor-related tumour induction in that the full range of tumour development was observed – liver hypertrophy (and likely hyperplasia (which was not reported, albeit assumed by Industry) as first step, foci and masses, adenomas and carcinomas in the liver. Incidences of carcinomas (alone) were 4% at 2000 ppm and 2% at 8000 ppm were not dose-related. However at this low range of percentage this does not compromise the concern resulting from the total numbers of liver tumours and putative precursor lesions or from the adenomas (alone).

- Liver hypertrophy may be considered as an adaptive effect if it is caused by induction of enzyme activities; if it is not associated to any other liver toxicity; and if it is a transient

phenomenon, which is fully reversible. Chronic rat studies on metazachlor demonstrated some indications of liver cell toxicity and persistence of liver hypertrophy. Thus taking the spectrum of non-neoplastic liver lesions observed into account the liver effects were not considered as adaptive.

- Intralobular degenerative lesions commonly considered as one putative mode of action leading to hypertrophic or hyperplastic responses were not consistently found across rat carcinogenicity studies. Degenerated (ballooning) hepatocytes were observed in another 2-year study on (Sprague-Dawley) rats at 6000 ppm (Hunter et al., 1983a). Marked increase in gamma-glutamyl transferase as seen in Wistar rats (+300/242% in males/females) (Krishnappa (2002) indicates hepatocyte dysfunction.

- Liver cell hypertrophy was not reported across all repeated dose studies in the rat. However, increased liver weight observed in oral 28-day and 90-day studies in Wistar and Sprague-Dawley rats surrogates early hypertrophic effect and/or proliferative/hyperplastic effect. In a single study where hepatocytic hypertrophy was reported, liver weight increase began at lower doses than hypertrophy.The LOAEL for significant liver weight increase was 1250 ppm (98 mg/kg/day, 90-day study in Wistar rats). All studies demonstrated that the size of weight gain was dose-related in rats.

- The carcinogenicity study on Sprague-Dawley rats (Hunter et al., 1983a) revealed some liver tumours at 2000 and 6000 ppm (data see Table below). The rates of liver adenomas in dose groups (4% in high dose males and 2% in females of mid and high dose groups compared to 0% and 2% in male and female control groups) were lower than in Krishnappa study. For evaluation of these data it should be regarded that the highest dosage (6000 ppm = 226/272 mg/kg bw/d in m/f) was lower compared to those of the Krishnappa study (8000 ppm = 361/442 mg/kg bw/d in m/f).

- The occurrence of 2-4% of liver carcinomas in all control and dose groups of male Sprague-Dawley rats and in 2% of the female control groups raises some uncertainty about the interpretation of the Hunter studies. In conclusion, these carcinogenicity studies do not give supportive evidence on liver carcinogenicity. However, the results of the Hunter studies do not invalidate the outcome of the Krishnappa study in Wistar rats.

- Although regarding lack of supporting evidence from the Sprague-Dawley studies (Hunter et al., 1983a,b), no clear sign of a strain-specific response could be identified by comparison of the two rat carcinogenicity studies since target organs and major (non-tumour) findings in the liver were comparable in both rat strains.

- Liver tumours were re-evaluated in 2008 by internal pathologists and by an independent Pathology Working Group (PWG) (Wiemann and Kaufmann, 2010a (Reference 25 in BD) according to the current WHO criteria. A slightly higher incidence of carcinomas in the top dose and a lower incidence of adenomas in the mid and high dose group of female Wistar rats was reported. Lower numbers in adenomas were partly explainable by reporting. (If an animal bears an adenoma and a carcinoma in the liver, the original evaluation contained separate entries, whereas the re-evaluation reported only the carcinoma.)

Liver tumours in female Wistar rats

Review	Females			
Dose (ppm)	0	200	2000	8000
Hepatocellular adenoma				
Original	1 (2 %)	0	1 (2 %)	8 (16%)
Internal	1 (2 %)	0	1 (2 %)	6 (12%)
PWG	1 (2 %)	0	0	6 (12%)
Historical control (internal) 1.13 % (0-6%) Dates: 08/96-09/05 RITA Database 0.9% (0-14%) Date: 01/94-02/05				
Hepatocellular carcinoma				
Original	0	0	2 (4 %)	1 (2 %)
Internal	0	0	2 (4 %)	2 (4 %)
PWG	0	0	2 (4 %)	2 (4 %)
Historical control (internal) 0 % Dates: 08/96-09/05 RITA Database 0.7% (0-4%) Date: 01/94-02/05				
Combined (adenoma/ carcinoma)				
Original	1 (2 %)	0	3 (6 %)	9 (18%)
Internal	1 (2 %)	0	3 (6 %)	8 (16%)
PWG	1 (2 %)	0	2 (4 %)	8 (16%)

- Increases in liver tumours in Sprague-Dawley rats were weak, increases were reported to be of no significance in the Fisher exact test and the Cochron Armitage trend test. Thus, no clear treatment-related effect was observed in the study of Hunter et al. (1993a) and Hunter et al. (1983b).

Liver tumours in male Sprague-Dawley rats

	Males					
Dose (ppm)	0		*100*	500	2000	6000
Hepatocellular adenoma						
Original	0	*2 (4 %)*	*0*	0	0	2 (4 %)
Internal	1 (2 %)	*1 (2 %)*	*0*	0	0	2 (4 %)
PWG	2 (4 %)	*1 (2 %)*	*0*	0	0	2 (4 %)
Historical control (internal) 1.13% (0-4%) Date: 03/78-10/84, RITA Database 2.5% (0-12%) Date: 09/83-10/02						
Hepatocellular carcinoma						
Original	2 (4 %)	*1(2 %)*	*1(2 %)*	1 (2 %)	2 (4 %)	2 (4 %)
Internal	2 (4 %)	*2 (4 %)*	*1 (2 %)*	1 (2 %)	2 (4 %)	2 (4 %)
PWG	0	*1(2 %)*	*1(2 %)*	1 (2 %)	2 (4 %)	2 (4 %)
Historical control (internal): 1.97% (0-6%) Date: 03/78-10/84 RITA Database 2.7% (0-8%) Date: 09/83-10/02						
Combined (adenoma/ carcinoma)						
Original	2 (4 %)	*3 (6 %)*	*1 (2 %)*	1 (2 %)	2 (4 %)	4 (8 %)
Internal	3 (6 %)	*3 (6 %)*	*1 (2 %)*	1 (2 %)	2 (4 %)	4 (8 %)
PWG	2 (4 %)	*2 (4 %)*	*1 (2 %)*	1 (2 %)	2 (4 %)	4 (8 %)

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, which is a supplementary study with identical conditions conducted 6 months later than the main study on 0 ppm and 100 ppm metazachlor.

- With respect to liver tumours in rat carcinogenicity study on metazachlor results of the peer-review were not markedly different from the original outcome. Overall, the review by internal pathologists and by the PWG confirmed the original results. The PWG themselves concluded that there might be a small treatment-related effect in Wistar rats (Wiemann and Kaufmann, 2010a, Reference 25 in BD). No significant indication for a treatment-related effect was seen in the liver of Sprague-Dawley rats treated with doses up to 6000 ppm.

- RAC appreciates the peer-reviewing of original histopathological evaluations by working groups of experienced experts. In studies with inconsistent or border-line results a peer-review of blinded slides could facilitate final conclusion on treatment-related adverse effects. Also in cases where terminology of tumour findings has markedly developed since the original evaluation of a early study, peer-reviewing is a valuable instrument. In cases where only selected sets of tissue slides were re-read and re-evaluations significantly diverge from original outcome, problems in interpretation of different outcomes will come up.

- In line with the comment from the Belgium MS it is stressed that only selected slides with neoplastic finding were reviewed. With respect to the liver all available slides of female Wistar rats and male Sprague-Dawley rats were re-evaluated. A complete re-evaluation of liver slides of all animals including males and females of all groups is recommended for future work.

It should be mentioned that it was the aim of Industry that the PWG should provide expert opinion on the discrepancies between original and internal re-evaluation. It was the intention of Industry to present the results of the PWG as the final outcome.
It is the opinion of RAC that discrepancy among original and internal re-evaluation is little and of no relevance for conclusion. Re-reading of the PWG confirmed earlier findings except for minor differences. Re-evaluation of selected slides in internal and PWG reviews limits the acceptability as final results.

Mode of action
Industry considers benign liver adenomas of female Wistar rats at the dose of 8000 ppm to be treatment-related but not relevant for humans based on a phenobarbital-like enzyme induction mediated by CAR (Constitutive Androstane Receptor) activation (Annex 2_RCOM-metazachlor_June 2010.doc). The marked induction of PROD1 (190fold) and BROD2 (116fold) at simultaneously low induction activity of EROD3 was considered to reflect the characteristic effects of phenobarbital on CYP2B-enzymes inducer activities characteristic for phenobarbital (Buesen, 2009a, 2010).

Enzyme activities in male rats were low (induction <10fold). In case enzyme induction is the relevant mode of action for tumour growth the low induction rates in male Wistar rats would be in line with low rate of liver tumours in this sex.

[1] PROD Pentoxyresorufin-o-dependentylase (CYP2B)
[2] BROD benoxyresorufin-o-debenzylase (CYP2B, CYP3A)
[3] EROD ethoxyresorufin-o-deethylase (CYP1A)

Li and Wang (2010) postulated that expression of rat CYP2b was mediated by activation of CAR. In fact, CAR protein was increased in liver nucleus from rats treated 3 and 7 days with with 8000 ppm metazachlor or 500 ppm phenobarbital (Li and Wang, 2010).

- In isolated hepatocytes of male Wistar rats, a weak activation of CYP2B1 expression was observed at metazachlor concentrations of 0.2-100 µM (2-fold and 16-fold, respectively) in comparision to 500-fold increased expression induced by phenobarbital at 1 mM (Neuschafer-Rube amd Puschel, 2010). The maximum expression was reported to be reached at 100 µM phenobarbitoal (not tested). As no cytotoxicity was observed at 10 µM metazachlor and viability was reduced to about 80% at 100 µM, interpretation of metazachlor findings remains unclear. A weak (1.5fold) activation of the (wild-type) CAR reporter gene was observed at (cytotoxic) concentration of 100 µM metazachlor. In the view of RAC the relevance of these findings in male Wistar rats (which did not show increased liver tumours) is equivocal. With respect to the postulated similarity to phenobarbital it is worth to note the cytotoxic effect of metazachlor, which was absent up to 1 mM phenobarbital.

- Increased cell proliferation is identified as an early event, which could contribute to the development of hyperplasia (foci) and adenomas. In an S-phase response study in Wistar rats receiving 200 ppm (13 mg/kg/day) and 8000 ppm (552-682 mg/kg/day) metazachlor with diet for up to 28 days revealed increased cell proliferative activity from day 3 onwards at 8000 ppm (Day 3: 8fold, Day 7: 12-fold, Day 14:15fold, Day 28: 6fold) (Buesen et al. 2010, see Annex II in BD). No significant response was seen at 200 ppm. No other data are available to establish dose-responses for liver cell proliferation at doses <8000 ppm and >200 ppm. No conclusion on the persistence and progression could be drawn, since no cell proliferation data are available for time periods after week 4.

  Phenobarbital has been shown to induce a (transient) increase in cell proliferation in liver cells of rats and mice (Whysner et al., 1996, reference 22 in BD). In spite of the data gaps described above, the fact that metazachlor enhances cell proliferative activity in liver cell is in line with the assumption of phenobarbital-like effects, but it does not give specific evidence for the same mechanisms behind the cell proliferation.

- The dossier submitter concluded that supplementary studies were not persuasive to demonstrate phenobarbital-like effects. Doubts for this mode of action are raised by the fact that a similar effect was not observed in mice, although they are the more sensitive species to phenobarbital-induced liver tumours. Concern is also raised by the fact that metazachlor was shown to be toxic to isolated rat liver cells whereas phenobarbital was not (Nuschafer-Rube and Puschel, 2010).

- In 2005, ILSI published its view that phenobarbital-like MOA for carcinogenic responses is deemed as non-relevant for humans (Holsapple et al. 2006, reference 21 in BD). Although ILSI indicated that there are data gaps it was concluded from patients receiving Phenobarbital for many years at doses producing plasma concentrations similar to those following a carcinogenic dose in rodents, there is no evidence of a hepatocarcinogenic effect. IARC concluded Phenobarbital as *possibly carcinogenic to humans (Group 2B)* according to their criteria based on inadequate evidence in humans and sufficient evidence in experimental animas (IARC, 2001, reference 23 in BD).

- At present, there are no established criteria for regulatory acceptance of this mode of action as of non-relevance for humans. Also, international agreement been not been reached that the effects of phenobarbital are not relevant for humans. Also it is not referred as mechanism of tumour formation that is accepted as non-relevant for humans (Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures, Chapter 3.6.2.3.2).

  Interestingly, phenobarbital (CAS 50-06-6) and (desoxyphenobarbital (-Ethyl-5-phenyldihydropyrimidin-4,6-dion (IUPAC) CAS No. 125-33-7), which is a prodrug that is metabolized to phenobarbital is classified on a voluntary basis as Carcinogen Cat 3, R40 (e.g., see www.sigmaaldrich.com).

- With respect to the comparison with phenobarbital uncertainties remain:

  Phenobarbital is suspected to activate other nuclear receptors in addition to CAR (e.g. the pregnane X receptor (PXR) (Holsapple et al., 2006) and has been shown to inhibit intercellular communication in hepatocytes (IARC, 2001).

  In spite of assumptions that there are phenobarbital-like effects (like CAR-activation related Cyp2B-induction, hypertrophy, liver tumours), uncertainties remain at the level of targeted genes. Transcriptomic analysis of liver cell RNA of mice receiving phenobarbital or conazoles, for which a similarity to phenobarbital-like CAR-mediated Cyp2B induction and hepatocarcinogenesis in mice was shown, revealed significant differences in gene expression. (Nesnow et al., 2009, reference 22 in BD). Microarray transcriptional studies to identify activated genes and pathways underlying the proliferative processes and to confirm similarities among phenobarbital and metazachlor are not available.

- In conclusion, metazachlor appears to have potential to activate CYP2B enzymes, is capable to activate CAR and stimulates proliferation of rat liver cells. It is found that there are some similarities to a phenobarbital-like response. However there are also some inconsistencies (lack of tumour response in mice, indications on cytotoxicity) and data are not yet sufficient to conclude that CYP mediated CAR activation is the only critical key event. A mode of action was not unanimously identified for the liver tumours and in conclusion the observed induction of liver tumours could not be ruled out as of no relevance for humans.

Tumour responses at other sites

Thyroid
Parafollicular (C-cell) tumour

Increases in C-cell tumours were observed in Sprague-Dawley (males only) receiving 2000 and 6000 ppm metazachlor. The tumour response for this type of tumour was not observed in Wistar rats up to 8000 ppm. The number of adenomas was slightly increased in males of the mid and high dose groups, there was also an increased incidence of carcinoma (Hunter et al., 1983a). However, this was lower, even at the top dose, than the incidence in the control group (16%) from the second Hunter study initiated six months later in the same laboratory. Thus, it remains

uncertain whether the increase in carcinomas should be considered to be treatment related. Overall, it is considered unlikely that the slight increase in benign adenomas in one sex and one strain is a treatment-related effect of metazachlor.

Thyroid C-cell tumors in Sprague-Dawley rats

Dose level (ppm)							
Males				Females			
0	500	2000	6000	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 tumours

Increases in these tumours (adenomas and carcinomas) were observed in Sprague-Dawley rats at 2000 ppm and 6000 ppm metazachlor, but not in Wistar rats at diet concentrations up to 8000 ppm. The incidence was well within the historical control range. Although a slight dose related non-significant 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 (adenoma 2% and carcinoma 4% in control males, see Appendix 1 of BD). 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. These increases were 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.

Phenobarbital was shown to increase pituitary thyroid-stimulating hormone in response to increased thyroid gland glucuronidation and biliary excretion in rats (IARC, 2001, reference 23 in BD). In case of a PB-like mode of action, increased incidences of thyroid gland tumours can be expected for this species. The absence of induced follicular tumours by metazachlor treatment and any change of T3/T4 levels in rats questions the hypothesis that the mode of action is PB-like.

Thyroid gland tumours in Sprague-Dawley rats

Dose level (ppm)							
Males				Females			
0	500	2000	6000	0	500	2000	6000
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%]							

Testis
Interstitial cells (Leydig cells) adenoma

A slightly higher incidence of these tumours were observed in Sprague-Dawley rats receiving the high dose, but the increase did not gain significance in comparison to low control values of 0 or 2% in both Hunter studies. No tumour response was observed in testes of Wistar rats. This increased incidence was also within the laboratory historical control range and is, therefore, not considered treatment related.

Testis tumours in Sprague-Dawley rats

Dose level (ppm)							
Males				Females			
0	500	2000	6000	0	500	2000	6000
Interstitial cell adenoma (leydig cells)							
1 (2%)	1 (3%0	1 (2%)	4 (7%)				
[historical control range (males)] [0-16%; mean 5.7 %]							

Summary of rat data
In the three available carcinogenicity studies in the rat, metazachlor was shown to have a carcinogenic effect in the liver of female Wistar rats (adenomas or combined adenomas and carcinomas). All other tumours observed are considered unlikely to be treatment related.

In the **mouse**, metazachlor appeared to have a weak carcinogenic effect in the kidney.

Kidney

- The 2-year study in CD-1 mice (Barnard et al., 1983) was identified as critical for the concern on kidney carcinogenicity.

- Benign kidney tumours were observed in dose groups, but the effect was inconsistent between strains and sexes (see Kumar, 2003). Increases in incidences of renal cortical adenomas occurred in male CD-1 mice only and were relatively small, but appeared to be dose-related (0%, 2%, 6%, 8% in controls, 200, 700 and 2500 ppm, 15, 154 und 578 mg/kg bw/d) within this bioassay.

  Kidney tumours in male CD-1 mice

	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.

- Arguments have been raised that tumour incidences were within the published ranges for historical controls. In a weight of evidence analysis control data on internal groups that are valid have priority above published historical data, in particular for non in-house historical data in the absence of a close time window.

- Metazachlor does not appear to be a genotoxic substance. Indications on non-genotoxic modes of action were not identified. Indications such as cytotoxicity or regeneration (basophilia) were neither observed in chronic and subacute studies on CD-1 mice nor in chronic studies on Swiss mice (Kumar, 2003).

- There was no evidence on cytotoxicity or increased mitotic rates (Re-evaluation by Hard, 2009, Reference 24 in BD). A S-phase response study in CD-1 mice revealed a slightly accelerated cell proliferation from 200 ppm. However, the response was not dose-related and minor (2.5 fold increase) at 2500 ppm at day 90 (Hard, 2010[4]). The only treatment-related non-neoplastic finding was elevated kidney weight in mid and high dose male (Swiss) mice that was not associated with a tumour response (Kumar, 2003). Marked increases in kidney weight were also seen in cancer studies on Wistar rats and Sprague-Dawley rats with no associated tumours in the kidney.

- Some inconsistency on the association of kidney tumours to metazachlor treatment were given by the absence of kidney tumours in the second mouse strain (Kumar, 2003) where males received a much higher diet concentration (4000 ppm ≈ 578 mg/kg/day compared to 2500 ppm ≈ 252 mg/kg/day in the study of Barnard et al., 1983), However, biological variability in animal studies are well known and disregarding positive tumour data could not be justified by the presence of a negative study only.

- No increases in kidney tumours were observed in <u>rat</u> carcinogenicity studies.

- Facts clarifying the mode of carcinogenic actions and indicating that the mechanisms causing kidney tumours were of non-relevance for humans could not be demonstrated. Data suggested a mode of action based on sustained toxicity and regenerative proliferative activity was unlikely.
  Industry considered kidney tumours in CD-1mice as not related to the treatment, the PWG concluded that increases in kidney tumours were unlikely to be treatment-related.

- Relevance for humans could only be denied if suitable data demonstrate that the mode of action has been identified and is not significant for humans.

Liver

[4] Hard GC (2010) Expert Re-examination of Quantitative Pathology Assesment of Proximal Tubule Cell Poliferation Activity in Kidneys of Mice Administered Metazachlor in the Diet for 7, 28, and 90 days. Final Report March 26, 2010, BASF DocID 2010/1054128.

A slightly increased rate of liver adenoma was observed in the 2-year study in CD-1 females (Barnard et al., 1983). Regarding the original evaluation the effect appeared to be dose-related; considering the re-analysis of PWG an effect could only be seen at the high dose (273 mg/kg bw/d).

Liver tumours in female CD-1 mice

	Females			
Dose (ppm)	0	200	700	2500
Hepatocellular adenoma				
Original	0	0	1 (2%)	3 (6%)
Internal	0	0	1 (2%)	3(6 %)*
PWG	1 (2 %)	0	1 (2%)	4 (8 %)
Historical control (06/78 – 10/84) 3.49 % (0-9.8%)				
Hepatocellular carcinoma				
Original	0	1 (2 %)	0	1 (2 %)
Internal	0	1 (2 %)	0	1(2 %)**
PWG	0	1 (2 %)	0	0
Historical control (06/78-10/84) 1.14 (0-4 %)				
Combined adenoma/ carcinoma				
Original	0	1 (2 %)	1 (2 %)	4 (8 %)
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.

The dossier submitter considered the (non-significant) increase in adenomas as by chance finding since the incidences were still within the historical range. The incidences in the control females and in low dose females are low, females of this strain used did not show high spontaneous rates. The upper limit of historical incidences should therefore not be used for valid controls to explain increased tumour rates.
Incidences of liver adenomas (16-25%) and carcinomas (22-30%) were high in male CD-1 mice of control and dose groups without any clear dose-response relationship.
Overall it appears questionable whether the increased incidence at the high dose group of females should be interpreted to be treatment-related. Due to this uncertainty the concern from the low increase in high dose females is not sufficient for classification.

Tumour responses at other sites

Increased rates of tumours were also found in the urinary bladder and the lymphoreticular system. However, increases were either very low (transitional cell papilloma) or could not attributed to metazachlor due to high spontaneous rates (malignant lymphomas). With respect to the bladder tumours supplementary studies did not reveal indications on microcrystallisation in the rat or mice urinary system (Buesen et al., 2009a and 2009d).

Summary of mouse data
In a carcinogenicity study on Swiss mice, metazachlor appeared to have a weak carcinogenic effect in the kidney of male mice. Only benign tumours were observed.
A treatment-related effect can not be excluded for the kidney tumours, however the association is considered to be weak. Other tumours observed in Swiss mice and in a carcinogenicity study on CD-1 mice are considered unlikely to be treatment related.

Overall conclusion on carcinogenicity

On the basis of increased tumour rates in two species and in the liver of rats and the kidney of mice and considering the fact that mode of actions were not identified and that absence of relevance of humans could not be confirmed, it is the opinion of RAC that classification for carcinogenicity is justified for metazachlor.

The major concern is from treatment-related liver tumours in female rats; weak tumour responses in the kidney of male CD-1 mice is considered to give supporting evidence since treatment-relationship could not be excluded.
RAC recognises that the overall tumour incidences were relatively small (4 (8%) in high dose mice vs. 0 in controls) and that there is lack of corresponding tumour finding in female animals and in another strain tested.
With respect to the carcinogenic potential in the rat liver, there is evidence on non-genotoxic mechanisms that bear similarities to a phenobarbital-like mode of action. However, inconsistencies with respect to the mouse and data gaps remain. Finally, the tumour responses could not be attributed to modes of action that would disclaim any relevance for humans.

In accordance with the criteria in CLP Regulation EC/1272/2008 classification in category 1A for carcinogenicity is not justified (accordingly category 1 in Directive 67/548/EEC) 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 1B or category 2.

Since increased tumours have been seen in two species, a simple argument for category 1B classification can be made. However, on consideration of the available data, there are a number of factors that indicate classification in category 2 would be more appropriate. Most significantly, there is the lack of genotoxicity seen with metazachlor in in-vitro and in-vivo studies. In the RAC's view a treatment-related tumour response could not be ruled out for the mouse, but it is also possible that the benign tumours in the kidney are chance observations.

In view of these considerations, RAC follows the proposal of the dossier submitter that the available evidence from liver tumours in the rat is deemed to best match the criteria for classification as a category 2 according to Regulation EC/1272/2008, and category 3 carcinogen according to Dir. 67/438/EEC.

There are no grounds to draw attention to a particular route of exposure on the label.

Toxicity for reproduction

Effects on fertility

Fertility effects of metazachlor are considered to be secondary to reduced food consumption and lower body weights. RAC agreed that no classification is proposed.

Developmental toxicity

Overall, there was no evidence of a direct adverse effect on development and no classification is proposed.

Environmental hazards
Only the aquatic compartment is relevant to this type of dossier.

Dossiers submitter's proposal for environmental hazard classification

- Aquatic Acute 1 (H400: very toxic to aquatic life) (CLP regulation) and R50/53 (Directive 67/548/EEC)
- Aquatic Chronic 1 (H410: very toxic to aquatic life with long lasting effects) (CLP regulation) and R50/53 (Directive 67/548/EEC)

The acute and the chronic classification categories are applied independently, according to CLP regulation.

Scientific evidence
Fate and behaviour of metazachlor in the environment was characterised by hydrolysis, photolysis, biodegradation and bioaccumulation.
Effects of metazachlor on aquatic life were assessed by studies reviewed and verified under Directive 91/414/EEC and is provided in the Pesticide Draft Assessment Report (DAR) which is attached to the IUCLID 5 dossier. Aquatic ecotoxicity data are available for metazachlor and for its metabolites BH 479-8, BH 479-9, BH 479-11 and BH 479-12, which are proven in all tests, being less ecotoxic than metazachlor. As a consequence the CLP classification is based on the hazard of metazachlor only.
Three trophic levels of the relevant surface-water ecosystem are: fish, invertebrates, algae/plants.

Fish studies
Based on four GLP acute fish toxicity (OECD Guideline 203) tests results and two 28-days sub-lethal fish toxicity studies (OECD Guideline 204) the lowest effect values measured by *Oncorhynchus mykiss*

The lowest **acute toxicity** result on fish	96-h LC_{50}:	**8.5** mg/l
The lowest **chronic toxicity** result on fish	28-days NOEC:	**2.15** mg/l

Aquatic invertebrates
Based on two short term static GLP 48-hour acute toxicity (OECD Guideline 202) and two long term semi-static GLP 21-day sub-lethal toxicity studies (EEC Guideline XI/681/86 and OECD Guideline 211) to *Daphnia magna* (water flea) the lowest effect values

The lowest **acute toxicity** result on *Daphnia magna*	48-h EC50:	**33** mg/l
The lowest **chronic toxicity** (reproduction) on *D. magna*	21-days NOEC:	**0.1** mg/l

Algae
Results of GLP static algal growth inhibition studies following OECD Guideline 201 using four algal species, which from *Scenedesmus subspicatus* (green alga) proved to be the most sensitive

The lowest **acute toxicity** (growth rate) result	72-h ErC_{50}:	**0.031** mg/l
The lowest **chronic toxicity** (growth rate) result	72-h NOErC:	**0.0018** mg/l

Aquatic plants
Three GLP growth inhibition studies are available following ASTM guideline E 1415-91 and EPA guidelines. *Lemna gibba* (duck weed*)* is highly sensitive water plant.

The lowest **acute toxicity** result on *Lemna gibba*	7-d ErC_{50}:	**0.0071** mg/l
The lowest **chronic toxicity** result on *Lemna gibba*	7-d NOErC:	**0.000193** mg/l

Degradability

Hydrolysis
Based on OECD Guideline 111 DT_{50} values at 20°C is 629 days, it means that metazachlor is hydrolytically stable under environmentally relevant pH and temperature conditions.
Photolysis
Aquatic photolysis study is not available, from molar light absorption results direct aqueous photolysis in the environment is not considered to occur.
Biodegradation
In a respirometric ready biodegradation study 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.
Two aerobic water/sediment **simulation** studies assessed the fate of metazachlor following SETAC guidelines, EPA guideline 162-4 and the BBA IV 5-1 guideline, using radiolabelled metazachlor. Results of the study show a decrease of metazachlor (radioactivity) in water, but an increase of radioactivity in sediment. No or very low radioactive CO_2 was detected, that means that no mineralization (biodegradation to CO_2) is going on in water or sediment. Carbon dioxide was not detected until 99 days. The highest CO_2 measurement was 1.3 % of the applied radioactivity on day 121.
Conclusion: both the screening and simulation tests proved metazachlor being not ready biodegradable.

Potential for bioaccumulation
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.

Public consultation
There was no disagreement on aquatic hazard during public consultation.

Comparison of available aquatic toxicity information with the criteria for each hazard class (Annex I, of the CLP Regulation)

<u>Classification according to the current CLP criteria</u>

<u>Under the current CLP Regulation metazachlor fulfils</u> the criteria for classification as Acute Category 1 (H400) and Chronic Category 1 (H410) based on the lowest reported acute aquatic toxicity value which is clearly below the threshold value of 1mg/l (7-d ErC_{50} = 0.0071 mg a.s./l for *Lemna gibba*) and its property as non-rapidly degradable substance. It was also shown that it is stable at environmentally relevant conditions and does not photodegrade in the environment.

M-factor is based on the lowest acute toxicity value (*Lemna gibba* (0.0071mg/l) which is, according to Table 4.1.3 in Annex I to CLP, in the range of 0.001 < 0.0071 < 0.01 mg/l resulting an M-factor 100.

<u>Classification according to the 2nd ATP criteria</u>

The 2nd ATP to the CLP Regulation will change the criteria for environmental hazard classification and after its publication (1st quarter of 2011) the criteria consider specific M-

factors for acute and chronic toxicities. Therefore, the classification according to the 2nd ATP criteria is given below.

Acute aquatic hazard

For metazachlor the lowest algal and aquatic plants effects value is a 7-d ErC_{50} = 0.0071 mg a.s./l for *Lemna gibba* based on mean measured concentrations. This concentration is below the threshold value 1 mg/l.

According to the low toxic concentration of metazachlor, it is classified as Category Acute 1 (H400). The lowest acute toxicity value: ErC = 0.0071, being between 0.001< 0.0071 < 0.01 mg/l, results M-factor (Acute) = 100.

- **Category Acute 1 (H400), M-factor (Acute) = 100**

Long-term aquatic hazard

For metazachlor the lowest chronic aquatic effect value (in this case NOErC) was measured in *L. gibba* (0.000193 mg/l). This values is below the set threshold (non-rapidly degradable substance) 0.1 mg/l. It is hydrolytically stable under environmentally relevant pH and temperature conditions and not considered to undergo photodegradation in the environment. On the basis of a ready biodegradation study, it is not considered rapidly biodegradable. No CO_2 production was detected in simulation tests within 99 days. Metazachlor fulfils the criterion "not to undergo significant mineralisation (with less than 70%) over 28 days".

Taking into account all the information on aquatic chronic toxicity and being non-rapidly degradable, metazachlor belongs to Category Chronic 1. The lowest chronic toxicity value: NOErC = 0.000193 mg/l, being between: 0.0001< 0.000193 < 0.001 mg/l, results for non-rapidly degradable substance, M-factor (Chronic) = 100.

- **Category Chronic 1 (H410), M-factor (Chronic) = 100**

For highly toxic substances, having acute toxic concentration below 1 mg/l, and chronic toxicity below 0.1 mg/l (if non-rapidly degradable) an M-factor (multiplying-factor) shall be applied for the classification of the substance as component of a mixture, even at low concentration.

Additional information

The Background Document, attached as Annex 1, gives the detailed scientific grounds for the Opinion.

ANNEXES:

Annex 1 Background Document (BD)[5]

Annex 2 Comments received on the CLH report, response to comments provided by the dossier submitter and rapporteurs' comments (excl. confidential information)

[5] The Background Document (BD) supporting the opinion contains scientific justifications for the CLH proposal. The BD is based on the CLH report prepared by a dossier submitter. The original CLH report may need to be changed as a result of the comments and contributions received during the public consultation(s) and the comments by and discussions in the Committees.