Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

metosulam (ISO)

EC number: N/A
CAS number: 139528-85-1

CLH-O-0000002525-76-03/F

Adopted

7 June 2013

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CLH-O-0000002525-76-03/F

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

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

Chemical name: metosulam (ISO);

EC number: N/A

CAS number: 139528-85-1

The proposal was submitted by **France** and received by the RAC on **14/05/2012.**

In this opinion, all classifications are given firstly in the form of CLP hazard classes and/or categories, the majority of which are consistent with the Globally Harmonised System (GHS) and secondly, according to the notation of 67/548/EEC, the Dangerous Substances Directive (DSD).

PROCESS FOR ADOPTION OF THE OPINION

France 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/harmonised-classification-and-labelling-consultation*** on **14/05/2012**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **28/06/2012**.

ADOPTION OF THE OPINION OF THE RAC

Rapporteur, appointed by RAC: **Thomasina Barron**

Co-rapporteur, appointed by RAC: **Riitta Leinonen**

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

The RAC opinion on the proposed harmonised classification and labelling was reached on **7 June 2013** and the comments received are compiled in Annex 2.

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

OPINION OF THE RAC

The RAC adopted the opinion that metosulam should be classified and labelled as follows:

Classification and labelling in accordance with the CLP Regulation

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard state-ment Code(s)	
Current Annex VI entry										
Dossier submitters proposal	616-214-00-8	metosulam (ISO); N-(2,6-dichloro-3-methylphenyl)-5,7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidine-2-sulfonamide	-	139528-85-1	Carc. 2 STOT RE 2 Aquatic Acute 1 Aquatic Chronic 1	H351 H373 H400 H410	GHS09 GHS08	H351 H373 H410		M-factor (acute): 1000 M-factor (chronic): 10
RAC opinion	616-214-00-8	metosulam (ISO); N-(2,6-dichloro-3-methylphenyl)-5,7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidine-2-sulfonamide	-	139528-85-1	Carc. 2 STOT RE 2 Aquatic Acute 1 Aquatic Chronic 1	H351 H373 (eyes, kidneys) H400 H410	GHS08 GHS09 Wng	H351 H373 (eyes, kidneys) H410		M=1000 M=100
Resulting Annex VI	616-214-00-8	metosulam (ISO); N-(2,6-dichloro-3-methylphenyl)-5,	-	139528-85-1	Carc. 2 STOT RE 2	H351 H373 (eyes,	GHS08 GHS09	H351 H373 (eyes,		

entry if agreed by COM		7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidine-2-sulfonamide			Aquatic Acute 1 Aquatic Chronic 1	kidneys) H400 H410	Wng	kidneys) H410		M=1000 M=100

Classification and labelling in accordance with the criteria of DSD

	Index No	International Chemical Identification	EC No	CAS No	Classification	Labelling	Concentration Limits
Current Annex VI entry	-						
Dossier submitters proposal	616-214-00-8	metosulam (ISO); N-(2,6-dichloro-3-methylphenyl)-5,7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidine-2-sulfonamide	-	139528-85-1	Carc. Cat. 3; R40 Xn; R48/22 N; R50/53	Xn; N R40-48/22-50/53	N; R50-53: C ≥ 0,025 % N; R51-53: 0,0025 % ≤ C < 0,025 % R52-53: 0,00025 % ≤ C < 0,0025 %
RAC opinion	616-214-00-8	metosulam (ISO); N-(2,6-dichloro-3-methylphenyl)-5,7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidine-2-sulfonamide	-	139528-85-1	Carc. Cat. 3; R40 Xn; R48/22 N; R50-53	Xn; N R: 40-48/22-50/53 S: (2-)36/37-46-60-61	N; R50-53: C ≥ 0,025 % N; R51-53: 0,0025 % ≤ C < 0,025 % R52-53: 0,00025 % ≤ C < 0,0025 %
Resulting Annex VI entry if agreed by COM	616-214-00-8	metosulam (ISO); N-(2,6-dichloro-3-methylphenyl)-5,7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidine-2-sulfonamide	-	139528-85-1	Carc. Cat. 3; R40 Xn; R48/22 N; R50-53	Xn; N R: 40-48/22-50/53 S: (2-)36/37-46-60-61	N; R50-53: C ≥ 0,025 % N; R51-53: 0,0025 % ≤ C < 0,025 % R52-53: 0,00025 % ≤ C < 0,0025 %

SCIENTIFIC GROUNDS FOR THE OPINION

RAC general comment

This opinion includes only those endpoints for which classification has been proposed by the DS and those for which discussion of classification was requested by RAC members

RAC evaluation of repeated dose toxicity (DSD) and specific target organ toxicity (CLP) – repeated exposure (STOT RE)

Summary of the Dossier submitter's (DS) proposal

The DS proposed to classify metosulam for specific target organ toxicity (STOT RE 2 – H373 (kidney, eye)) and for repeated toxicity R48/22 (DSD) on the basis of observed toxicity to the eye (dogs) and to the kidneys (rats, mice, dogs and rabbits), supported by an extensive data base of animal studies. The short-term oral toxicity of metosulam was evaluated in CD1 mice, Sprague Dawley and Long Evans rats, Beagle dogs, and rabbits for treatment durations of 2 to 52 weeks. A 3-week study was performed by repeated dermal applications in rabbits. The results of these investigations are summarised below.

Oral toxicity studies:

CD1 mice:
Metosulam was administered daily to CD1 mice by dietary admixture at 0, 100, 500, 1000, 2000 or 5000 mg/kg/day for 2 weeks, and 0, 250, 1000 or 2000 mg/kg/day for 13 weeks. Lower platelet counts (-23%) were observed in females and were also present as a trend in males treated at 2,000 mg/kg/day at the end of the 13-week treatment period. Hepatic centrolobular hypertrophy was observed with a dose and time-related severity in animals given the test item at doses > 1000 mg/kg/day. Slightly decreased liver vacuolation was also observed in both sexes at 5000 mg/kg/day for 2 weeks, and focal necrosis and inflammation of the liver in 2/10 males at 2000 mg/kg/day for 13 weeks. Slightly higher incidence of renal tubule degeneration/regeneration was observed in male mice at 2000 mg/kg/day for 13 weeks.

Rats:
Metosulam was administered daily to Sprague Dawley rats by dietary admixture at 0, 100, 500, 1000, 2000 or 5000 mg/kg/day for 2 weeks and 0, 10, 100, 500 or 1000 mg/kg/day for 13 weeks followed by a 4 week recovery period, and to Long Evans rats at 0, 1000 or 5000 mg/kg/day for 2 weeks.
No target organs were identified after 2-weeks of administration. The only adverse effect was lower body weight gains in Sprague Dawley rats treated at dose-levels higher than 100 mg/kg/day, associated with a decrease in diet palatability.
When administered for 13 weeks, diet supplemented with metosulam at nominal dose levels of 100 mg/kg/day and higher was unpalatable and reduced food consumption associated with reduced body weight gain were observed in males treated at > 100 mg/kg/day and females treated at > 500 mg/kg/day. However, the body weight gain difference failed to completely recover during the treatment free period and was therefore also attributed to toxic effects of the test item. Other adverse effects were related to the dysorexia, including minimally lower cholesterol, triglycerides, total proteins and albumin, and decreased amount of mesentery fat. The kidneys were identified as the main target organ with minimally lower urine specific gravity observed at urinalysis at the end of the treatment period in males given metosulam at > 500 mg/kg/day, higher creatinine and calcium blood levels observed at blood biochemistry investigation at the end of the treatment period in males given metosulam at > 500 mg/kg/day, minimally lower kidney weights (1000 mg/kg/day), and proximal renal tubule lesions including epithelial hypertrophy, increased basophilia (respectively in males and females, both 500-1000 mg/kg/day) and nuclear pleomorphism (both sexes, 100-1000 mg/kg/day). All these adverse effects were reversible at the end of the 4-week recovery period, with the exception of nuclear polymorphism of renal tubules.

Beagle dogs:
Metosulam was administered daily to Beagle dogs by dietary admixture at 0, 25, 100, 250, 500 and 1000 mg/kg/day for 2 weeks, 0, 5, 25 or 50 mg/kg/day for 13 weeks, and 0, 3, 10 or 37.5

mg/kg/day for 52 weeks. Deaths were observed at dose-levels ≥ 250 mg/kg/day, associated with clinical signs of weakness, and dose-related severe reduction of food intake causing body weight loss at doses ≥ 100 mg/kg/day.
Lower platelet counts (ca -30% when compared to controls) were observed in both sexes treated at 50 mg/kg/day for 13 weeks, and at 37.5 mg/kg/day for 52 weeks.
Ocular lesions, including retinal detachment, necrosis and atrophy were observed in animals from 100 mg/kg/day in 2-week studies, 50 mg/kg/day in the 13-week study, and 37.5 mg/kg/day in the 52-week study.
Renal lesions including tubular necrosis and mineralization, fibrosis, mononuclear aggregates and renal collecting duct degeneration were observed at dose-levels ≥ 100 mg/kg/day after 2 weeks of treatment, ≥ 25 mg/kg/day after 13 weeks of treatment, and 37.5 mg/kg/day after 52 weeks of treatment. These lesions were associated with lower urine specific gravity in both sexes treated at 50 mg/kg/day for 13 weeks, slightly higher creatinine blood levels in animals treated at 37.5 mg/kg/day for 52 weeks, and lower blood potassium levels in females treated at 37.5 mg/kg/day for 52 weeks and in both sexes treated at 50 mg/kg/day for 13 weeks. Diffuse urocystitis was also observed in females treated at 37.5 mg/kg/day for 52 weeks.
Hepatic lesions were also observed, including periportal aggregates of mononuclear cells in the liver of females treated at 50/mg/kg/day for 13 weeks, and mucin accumulation within exaggerated mucosal folds in the gall bladder in animals treated at 37.5 mg/kg/day for 52 weeks, both associated with higher alkaline phosphatase blood levels.

New Zealand white rabbits:
Oral administration of metosulam to female rabbits at dose-levels up to 1000 mg/kg/day for 2 weeks induced mortality preceded by soiling and anorexia, and subsequently lower body weight gains (600-1000 mg/kg/day), markedly higher (+91% relative) kidney weights in the surviving animal at 600 mg/kg/day, with renal tubular epithelial cell degeneration and necrosis at 300 (very slight, 1/3 animals) to 1000 mg/kg/day (slight to moderate, systematic), and focal necrosis and inflammation of the gallbladder at 600-1000 mg/kg/day. A NOAEL could therefore not be set; however the dose-level of 300 mg/kg/day was considered as a LOAEL based on slight renal lesions observed following histological examination.

Dermal toxicity studies:

New Zealand white rabbits:
Rabbits exposed by skin contact to metosulam at dose-levels of 100, 500 or 1,000 mg/kg/day 5 days/week for 3 weeks showed only very slight to slight epidermic hyperplasia (females treated at 100-1,000 mg/kg/day and males treated at 500-1,000 mg/kg/day) attributable to irritation caused by skin contact with the granular test item. There were therefore no significant treatment-related findings. The NOAEL was 1,000 mg/kg/day in both sexes.

Additional toxicology investigations:
No retinal lesions were observed in beagle dogs treated for 14 days by ocular topical application of metosulam, and no ocular lesions were found in cynomolgus monkeys treated for 6 weeks with metosulam at the dose-level of 100 mg/kg/day by oral route. In cynomolgus monkeys, the concentration of metosulam in serum, measured once, 1 hour after dosing on week 6, was 90.8 µg/mL, i.e. in the range of those observed in dogs (150 µg equivalent/mL including almost exclusively unchanged metosulam, at 4 hours after a single administration).

In conclusion, of the toxicity of metosulam was highest in dogs and lowest in CD1 mice and was mainly detected in post-mortem examinations, with limited functional impairment, except for reduced body weight gain and retinal detachment causing blindness in dogs. The target organs are the kidneys (mainly renal tubule degeneration/regeneration) in mice, rats, dogs and rabbits and the eyes (observed in dogs only) with severe lesions of retinal detachment and necrosis leading to blindness. In addition, but to a much lesser extent, the liver was also the target in mice and dogs, and the gallbladder in dogs and rabbits. These findings were not considered sufficient to support classification.

Comments received during public consultation
Two member states supported the classification proposal of the DS.

RAC assessment and comparison with the classification criteria

Renal lesions:
Metosulam induced renal degenerative lesions in dogs at the oral dose levels of 100 mg/kg/day, administered for 2 weeks, 25 and 50 mg/kg/day administered for 13 weeks, and 37.5 mg/kg/day administered for 1 year. Furthermore, this type of lesion was also observed at higher doses in repeated dose toxicity studies in rats (13-week, 2-generations & 24 month studies), rabbits (2-week and developmental studies) and mice (13-week study).

Ocular lesions:
Metosulam induced retinal detachment and necrosis in dogs at the oral dose levels of 100 mg/kg/day administered for 2 weeks, 50 mg/kg/day administered for 13 weeks, and 37.5 mg/kg/day administered for 1 year, but such lesions were absent in SD rats or CD1 mice in all oral route-studies. The oral absorption of metosulam has been shown to be similar in mice and dogs (20.6 and 19.2% at the dose-level of 100 mg/kg), while it is much higher in Sprague Dawley rats (males: 59.1%, females: 70.6% at the same dose-level). In all three species, the Tmax was 4 to 6 hours after dosing. After oral administration, metosulam was detected in large amounts in the eyes of dogs and especially in the optic nerve, iris, sclera and mostly the retina. Therefore, it appears that the ocular lesions induced by oral administration of metosulam to dogs, are species specific as they are not detected in any of the 4 other animal species/strains investigated. This may be related to the accumulation of metosulam in the tapetum and in the retina of the dog (Timchalk et al., 1992).
However, the mechanism of action is unknown. Consequently, relevance to humans remains unclear.

Comparison with CLP criteria
The guidance values refer to effects seen in a standard 90-day toxicity study conducted in rats. They can be used as a basis to extrapolate equivalent guidance values for toxicity studies of shorter or longer duration, using dose/exposure time extrapolation similar to Haber's rule for inhalation, which states essentially that the effective dose is directly proportional to the exposure concentration and the duration of exposure.

The effective doses seen in the different studies after conversion to 90 day equivalents using Haber's rule showed that the relevant renal and ocular lesions are observed within the equivalent of the guidance range of 10-100 mg/kg/d except for the oral 2-week study in Sprague-Dawley rats, in which the renal lesions are seen at the converted value of 5 mg/kg/d. This value would lead to a category 1 classification. However, based on the more substantial results of the two-generation reproduction (18-weeks administration) and the two-year chronic studies for this species/strain, metosulam would not be classified according to the same conversion rule.

As a consequence, metosulam should be classified as STOT-RE. 2 – H373 (kidney, eye).

Comparison with the DSD criteria:
Substances are classified as R48/22 when sufficiently serious damage (clear functional disturbance or morphological change which has toxicological significance), is likely to be caused by repeated or prolonged exposure. As a reference, substances are classified at least as harmful when these effects are observed at levels of the order of $\leqslant$ 50 mg/kg/day in an oral 90-day repeated-dose study conducted in rat.

Consequently, as the relevant renal and ocular lesions listed above, are observed ≤ 50 mg/kg/d when administered to the dog, in the sub-chronic studies (13 weeks & 1 year) and ≤ 150 mg/kg/day in the sub-acute studies, metosulam should be classified as Xn, R48/22 according to Directive 67/548/EEC.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier submitter's proposal

In vitro data: Technical metosulam had no mutagenic effect in an Ames test at dose-levels up to 100 µg/plate, without and with metabolic activation (Samson & Gollapudi, 1990). This test did not include the TA102 strain of *Salmonella typhymurium*, or any recommended strain of *E. coli*. The

lack of genotoxic effects of metosulam was investigated in the additional strains TA 102 (Herbold, 2007) and it exhibited no mutagenic effect with and without metabolic activation under the test conditions used.

Technical metosulam did not induce gene mutation at the HPRT locus in CHO and V79 cells, when tested at concentrations up to the limit of solubility and above. Although the V79/HGPRT test did not include a repetition assay with metabolic activation, its results are concordant with the CHO test and therefore acceptable.

Technical metosulam did not induce structural chromosome aberrations in V79 cells, when tested up and above its limit of solubility. It had no potential to induce chromosomal damages in cultured rat lymphocytes after incubation for 4 hours without and with metabolic activation up to the limit of solubility, and with harvest times at 24 and 48 hours.

In an unscheduled DNA synthesis test, metosulam did not induce significant changes in the nuclear labelling of primary rat hepatocytes when tested up to the limit of solubility in comparison to solvent controls.

In vivo data: When given as a single oral dose by gavage to male and female CD 1 mice, at the maximal dose-level of 5000 mg/kg, metosulam did not induce any cytotoxicity in the bone marrow cells and did not induce any significant increase in the frequencies of micronucleated bone marrow polychromatic erythrocytes. The blood levels of the test item were not measured in the study, but a separate pharmacokinetic study demonstrated that the absorption of metosulam in CD1 mice after oral administration was ca. 21% (see 4.1.1).

An additional comet assay (Wirnitzer, 2007, see BD) was performed with metosulam, by administration of a single oral dose by gavage at the maximal dose-level of 500 mg/kg, did not induce DNA damage in vivo in renal cells of male Sprague Dawley rats.

Comments received during public consultation

No comments on germ cell mutagenicity were received during public consultation

Assessment and comparison with the classification criteria

Considering that metosulam induced no genotoxic effect in *in vitro* and *in vivo* tests, no classification is required for metosulam under either CLP or the DSD.

RAC evaluation of carcinogenicity

Summary of the Dossier submitter's proposal

A proposal for classification for carcinogenicity was made by the DS on the basis of the data submitted in the EFSA draft risk assessment report (DAR). The long-term toxicity and carcinogenicity of metosulam was evaluated in rats and mice exposed orally to metosulam for 1.5 or 2 years and the results are summarised below.

Two year chronic toxicity/carcinogenicity study in rats:
Dietary administration of metosulam to Sprague Dawley rats at the concentrations of 5, 30 and 100 mg/kg/day for 2 years induced the following treatment-related effects in animals killed at the end of the 2nd year:
-higher WBC counts in high-dose males, with a slight shift from lymphocytes to neutrophils;
-lower urine specific gravity in high-dose males and in females treated at 30 and 100 mg/kg/day;
-at histopathology, higher frequency of non-neoplastic (but potentially pre-neoplastic) renal lesions: proximal tubule epithelial cell nuclear pleomorphism at 30 and 100 mg/kg/day, and (mainly tubular) epithelial hyperplasia in high-dose males;
-higher frequency of kidney nodules or masses in high-dose males (18/50);
-higher frequency of malignant tumors in high-dose males, and of renal neoplasms in both sexes in the high-dose group; this was due to basophilic renal cortex tumors, either adenomas (low frequency) or adenocarcinomas (high frequency in males, more than half metastatic).

The DS considered that the kidneys are the target organ for long-term oral toxicity in rats. Metosulam has a carcinogenic effect on kidneys in rats treated at 100 mg/kg/day, especially in males.

18-month carcinogenicity study in mice:
Dietary administration of metosulam to CD1 mice at 30, 300 and 1000 mg/kg/day for 18 months induced the following treatment-related effects in animals killed at the end of the treatment period:
-minimally lower kidney weights (males 17%, females 10%) in mice administered 1000 mg/kg/day;
-a minimally increased frequency of irregular renal cortical surface in females given 1000 mg/kg/day (10/50 compared to 2/50 in controls).

Metosulam had, according to the DS, a mild (no histopathologic lesions) long-term toxic effect on the kidneys at 1000 mg/kg/day but showed no carcinogenic potential in this mouse study.

Sensitivity to the long-term toxic effects of metosulam was higher in rats than in mice, and metosulam was only carcinogenic in rats. The only target organ was the kidney in both species.

The initial event in the development of renal toxicity induced by metosulam is the degeneration and subsequent necrosis of susceptible tubular epithelial cells. Following the initial injury, the mitotic activity of adjacent epithelial cells increases, as demonstrated by markedly increased 5-bromo-2-deoxyuridine (BrdU) incorporation (see study in SD rats, Yano *et al*, 1992, see BD). Epithelial cells which have recently undergone mitosis appear small and more basophilic than the unaffected epithelial cells and are consistent with the regenerative epithelial cells noted histologically in males and females. As these newer epithelial cells mature, their cytoplasm becomes more eosinophilic and nuclear pleomorphism becomes more apparent. Tubular epithelial cell necrosis and mitosis diminish with time, as the only indication of cell injury present after 13 weeks of exposure to metosulam was nuclear pleomorphism, epithelial cell hypertrophy and cytoplasmic basophilia of epithelial cells (see study in SD rats by Szabo & Grandjean, 1989, see BD).
After a 2 year treatment period, treatment-related renal cortical adenomas and adenocarcinomas were observed in 18/50 males, and 6/50 females given 100 mg/kg/day. The occurrence of tubular epithelial cell hyperplasia noted in the 2 year study paralleled the occurrence of renal tumours and may be the morphologic precursor of renal tumours. The sex difference in the incidence of renal tubular epithelial hyperplasia and tumours induced by the administration of metosulam to rats for up to 2 years is most likely related to the sex specific localization of renal lesions in the cortex of male rats and primarily to the medulla of female rats.

It was hypothesized by the DS that dose-levels of metosulam which do not cause tubular epithelial cell necrosis and regeneration would not be expected to cause epithelial cell pleomorphism, multifocal hyperplasia or tumours. This is supported by the finding that continuous administration of 5 mg/kg/day for up to 2 years did not lead to the formation of renal tumours in rats and by the lack of carcinogenic effect in mice, where no tubular epithelial cell necrosis and regeneration was observed.

Based on the lack of genotoxicity potential and on the clear evidence of epithelial cell necrosis and increased mitotic activity, The DS considers metosulam as a non-genotoxic carcinogen and proposes classification as Carc. 2 – H351 (Carc. Cat. 3; R40 according to DSD)**.**

Comments received during public consultation
Comments were received from two MS, both agreeing with the proposal of the DS.

RAC Assessment and comparison with the classification criteria
Comparison to Criteria:
Classification in Cat 1(CLP/DSD) is not required as there is no evidence for carcinogenicity in humans.

Classification in Cat 1B (CLP)or Cat 2 (DSD) are not considered appropriate as the evidence of carcinogenicity is restricted to a single experiment/species and to a single site, and as a threshold has been shown for the observation of tumours. In addition, there is no demonstrable genotoxicity potential, and on the additional study data showing that tubular epithelial cell necrosis and regeneration probably lead to the formation of renal tumours. Metosulam can be considered as a non-genotoxic carcinogen and should not be classified as category 1B Carcinogen (category 2 carcinogen according to DSD).

Substances are classified as a category 2 Carcinogen when evidence is obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations.

Consequently, the following elements are considered:

- After a 2 year treatment period, treatment related renal cortical adenomas and adenocarcinomas were observed in 18/50 males, and 6/50 females given 100 mg/kg/day.
- Sensitivity to the long-term toxic effects of metosulam was higher in rats than in mice, and metosulam was only carcinogenic in rats.
- The initial event in the development of renal toxicity induced by metosulam is degeneration and subsequent necrosis of susceptible tubular epithelial cells. Following the initial injury, mitotic activity of adjacent epithelial cells increases, as demonstrated by markedly increased BrdU incorporation (see study in SD rats, Yano et al, 1992)
- The occurrence of tubular epithelial cell hyperplasia noted in the 2 year study in rats paralleled the occurrence of renal tumours and may be the morphologic precursor of renal tumours. This does not appear to occur in cynomolgus monkeys.
- As mentioned above, dose-levels of metosulam which do not cause tubular epithelial cell necrosis and regeneration would not be expected to cause epithelial cell pleomorphism, multifocal hyperplasia or tumours.

Based on the observation of treatment related renal cortical adenomas and adenocarcinomas observed in males and females rats, the RAC agreed with the DS's proposal that metosulam should be classified as Carc. 2 – H351 (Carc. Cat. 3; R40 according to DSD).

RAC evaluation of environmental hazards

Summary of the Dossier submitter's proposal

Metosulam is currently not listed in Annex VI of the CLP Regulation. As the substance is unlikely to bioaccumulate, is not rapidly degradable according to CLP but readily degradable according to the DSD, and is very toxic to *Lemna minor* (acute ErC_{50}= 0.000789 mg/l, chronic NOEC = 0.00015 mg/l) the dossier submitter proposed to classify the substance as Aquatic Acute 1 (M=1000) and Aquatic Chronic 1 (M=10) according to CLP and N, R50-53 according to DSD with specific concentration limits of:
C≥0,025% N; R50-53; 0,0025%≤C<0,025% N; R51-53; 0,00025%≤C<0,0025% R52-53.

Degradation
A GLP-compliant hydrolysis study according to US EPA guidelines was run for 30 days. No hydrolysis was observed demonstrating that the substance is hydrolytically stable at pH 5, 7 and 9 at 25 °C.

There are two photolysis studies of metosulam available, one in water and one in soil. The first study was performed according to the UBA guideline is GLP-compliant andthe conclusion was that in pure water, photodegradation may contribute to the elimination of metosulam from the environment to a significant extent. However, in buffer solutions, in the presence of organic or inorganic ions, no absorption at > 396 wavelengths was observed. The second study was run according to an EPA guideline and was also GLP-compliant with a half-life for direct photodegradation of 31.1 days. No transformation products of metosulam were observed in significant amounts. In soil, after application of A-metosulam (aniline-[*UL*-^{14}C]-metosulam) to a silt soil, the degradation was slightly faster under irradiation. The degradation half-lives were 18.3 days under irradiation, and 42.3 days in the dark. Mineralization was low, reaching 2.9% AR

(applied radioactivity) of $^{14}CO_2$ in irradiated samples. Bound residues increased to 34.3% AR in irradiated soils and 18.4% AR in dark controls. Metosulam was the only compound detected in significant amounts in soil extracts. In addition to metosulam, 1.1 % of other radiolabelled extractable compounds were found in irradiated soil extracts.

There are two GLP-compliant screening studies on ready biodegradation of metosulam performed according to OECD 301D (Closed Bottle Test) and 301B (Modified Sturm Test) guidelines. In the Closed Bottle Test, oxygen was not consumed in bottles containing the test substance alone, while oxygen consumption in bottles containing sodium benzoate (66% of ThOD) demonstrated that the inoculum was viable under the test conditions. Degradation of benzoate was not essentially affected by the presence of metosulam (51% and 54% of the ThOD of benzoate). This indicates that metosulam was not readily biodegradable under the test conditions and at the tested concentrations (2 and 10 mg a.s./l) did not inhibit bacterial activity. In the Modified Sturm Test, CO_2 production by reaction mixtures (mineral salt medium, bacterial inoculum and the test substance) containing 10 mg/l and 20 mg/l metosulam was 0.5 and 2.7 mg after 28 days, which was equivalent to 1% and 3% of the theoretical CO_2. These results indicate that metosulam is not readily biodegradable. The dossier submitter concludes that metosulam is not readily biodegradable based on these test results.

In biologically active water sediment systems, metosulam had a half-life of 8 days in the water phase as well as in sediment. Degradation leads to the formation of three major metabolites: M01, M02 and M04 as follows:

Metabolites	Water	Sediment
M01	17.4% AR 60 DAT	15.7% AR 120 DAT
M02	17.2% AR 14 DAT	17.8% AR 14 DAT
M04	15.6% AR 14 DAT	4.2% AR 14 DAT

AR=applied radioactivity DAT=day after treatment

Bound residues	58.7 - 67.5% AR 120 DAT
Mineralization	3.6% AR 120 DAT

Substance	max DT50 in the total system	max DT90 in the total system
Metosulam	8.2 days	27.1
M01 (ATSA)	120 days	
M02 (7-OH-metosulam)	22.4 days	
M04 (5,7-OH-metosulam)	14.1 days	

Substance	Max DT50 Water	Max DT50 Sediment
Metosulam	7.5 days	31 days

Under aerobic conditions, metosulam was rapidly degraded in soils. The major metabolites were M01 (26.3%) and M02 (21.8%). A third soil metabolite, M03, was formed in amounts < 5%. Mineralisation was variable among soils (2-12% after 185 days), non-extractable residues reached high amounts (51.9-65.7%, 122 DAT). The degradation half-lives of metosulam ranged from 4.9 to 43.4 days, with a geometric mean of 10.6 days. M01 and M02 were rapidly degraded in soils under aerobic conditions, with geometric means of 54.9 and 2.2 days, respectively. After application to bare field soils, metosulam was almost exclusively distributed in the 10 cm superficial layer of soil. A first order kinetic dissipation rate allowed mean DT50 of 31.9 days and a mean DT90 of 104.2 days to be calculated.

The DS's conclusion on degradation is that metosulam is rapidly degradable according to the CLP Regulation and is based on the fact that according to the 2nd ATP of the CLP Regulation degradation products have to be considered in the assessment of rapid degradability. Considering that metosulam is significantly more toxic to algae and aquatic plants than to invertebrates and fish, the aquatic toxicity data for degradation products which are available only for algae and aquatic plants are considered sufficient to fully address their hazard profile for the aquatic environment. Since the degradation products do not fulfil the criteria for classification as

hazardous to the aquatic environment, primary biodegradation of metosulam is considered sufficient in the assessment of rapid degradability of this substance.

Bioaccumulation
Based on the log Kow values 1.8 (pH 4), 0.2 (pH7), -1.1 (pH 9) at 20 °C, metosulam has a low potential to bioaccumulate. The DS further reported a study on bioconcentration (OECD 305, reliability 1) which showed that there was no evidence of bioaccumulation of metosulam in fish tissues after exposure to the active substance. However, no more information on the test is given in the CLH Report.

Aquatic toxicity
The substance is a herbicide. There is information on short-term and long-term toxicity for fish, aquatic invertebrates, algae, and aquatic plant *Lemna minor*. According to the dossier submitter most of the tests were performed according to OECD or EPA guidelines and under GLP.

Table 1 Lowest acute aquatic toxicity values for each trophic level

Species	Test guideline	Test type	Result
Oncorhynchus mykiss	OECD 203, GLP	static	96 h LC_{50}: > 29.3 (measured conc.)
Crassostrea virginica	EPA 1988, GLP	flow-through	96h, EC_{50}: 87.7 mg/l (nominal)
Scenedesmus subspicatus	OECD 201, GLP	static	ErC_{50} 48 h: 0.17 mg/l (nominal) EbC_{50} 72 h: 0.075 mg/l (nominal)
Lemna minor	OECD 221, GLP	static	7d ErC_{50}: 0.000789 mg/l (TWA) 7 d EbC_{50}: 0.00230 mg/l (TWA)

Table 2 Lowest chronic aquatic toxicity values for each trophic level

Species	Test guideline	Test type	Result
Pimephales promelas	OECD 210, GLP	flow-through	NOEC: 4.24 (measured conc)
Daphnia magna	OECD 202, GLP	semi-static	21 d NOEC: 2.5 mg/l (nominal)
Scenedesmus subspicatus	OECD 201	static	72 h NOEC: 0.02 mg/l (nominal)
Lemna minor	OECD 221	static	7 d NOEC: 0.00015 mg/l (TWA)

The most sensitive species is clearly the aquatic plant *L. minor*, as evidenced by a study performed under GLP according to OECD 221 guideline. The nominal test concentrations were 0, 0.100, 0.320, 1.00, 3.20 and 10.0 µg/l. The measured concentration on day 7 ranged, however, from 24 to 36 % of corresponding nominal concentration, except for the lowest initial concentration at which measured values were below the limit of quantification (0.05 µg/l). According to the dossier submitter geometric mean concentrations wof <0.05, 0.05, 0.15, 0.51, 1.81, and 6.35 µg/l were calculated over the exposure period and the results are based on these measured concentrations. The EFSA DAR, however, states that the values are time weighted average (TWA) mean concentrations. The aquatic toxicity values based on these concentrations for *L. minor* are: 7-day ErC50 of 0.789 µg/l and a 7-day NOErC of 0.15 µg/l.

There are also toxicity data available for degradation products M01 (ATSA), M02 (7-OH metosulam) and M04 (5,7-OH metosulam) on algae and *L. minor*. For M01 the results for algae and *L. minor* are the same: 72-h ErC50 > 10 mg/l, NOErC 10 mg/l for algae and 7-day ErC_{50} > 10 mg/l, NOEC 10 mg/l for *L. minor*. For M02 and M04 *L. minor* was the most sensitive species with 7-day ErC_{50s} 19 and 7.95 mg/l and NOECs 3.2 and 1.8 mg/l, respectively.

Comments received during public consultation

Comments were received from one MS who agreed with the environmental classification proposal made by the dossier submitter.

Two MS asked for more information on the toxicity of metabolites, in order to substantiate that the degradation products are not classifiable, i.e. requesting the DS to show that the degradation products are not more toxic to fish and daphnia than the parent compound. Whilst the parent compound is most toxic to algae/aquatic plants, the dossier does not provide evidence that the degradation products are more toxic to algae/aquatic plants than to fish and crustaceans. The DS confirmed that there are no data available on daphnia and fish toxicity and explained that based on the toxicity values of the parent to fish and *Daphnia* and the fact that degradation products are much less toxic for algae and aquatic plants, toxicity values < 1 mg/l are not expected for the degradation products.

Three MS partially agreed with the proposed environmental classification but considered the substance as not rapidly degradable. Although the DT_{50} in aqueous simulations test is smaller than 16 days and 3 major non classifiable metabolites are formed, mineralisation only accounted for a maximum of 3.6% of applied radioactivity (AR) at day 120. in addition, no ultimate degradation is demonstrated in the soil study. In the EFSA DAR it is stated that in an aerobic degradation study in four soils, mineralisation occurred to a generally small extent, reaching 10% AR 122 days after treatment in the soil with the highest microbial activity. For field soils, a DT_{50} of 31.9 days was determined but no information was given on mineralisation. Based on this, the MS agreed with classifying as Aquatic acute 1, H400, M=1000 and Aquatic Chronic 1, H410. However, based on the substance being not rapidly degradable, the M factor for chronic toxicity should be 100. Classification according to the DSD should then be N, R50-53.

Another MS agreed that the metabolites are not classifiable but they believed more information was needed in order to conclude whether the DT_{50} means degradation or dissipation and how much of the parent compound is transformed into degradation products. According to the data, 60% AR is measured 120 days after treatment in bound residues. This would imply that a considerable amount of the parent compound does not undergo primary degradation but is in the form of bound residues. If so, the criterion for fast primary degradation is not met and the reason for considering the substance as rapidly degradable is absent. One MS thought that further explanation on the aquatic fate of the parent compound and its degradation products was needed. For example, do metabolites M01, M02 and M04 undergo degradation to produce further metabolites? The DS agreed with these comments and consequently that the rapid degradability of the substance is not fully demonstrated. The DS thus supported the new proposal for an M factor for chronic toxicity of 100.

An MS also requested to include the result of the BCF study in the CLH report instead of a general remark 'there was no evidence on bioaccumulation'. According to the DS there was no evidence of bioaccumulation of metosulam in fish tissues after exposure to the active substance for 96 hours at the actual concentration of 0.08 and 0.8 mg/l. The BCF could not be calculated as the radioactivity levels in fish were below the limit of quantification. Then it was assumed that the BCF is below 100.

Assessment and comparison with the classification criteria

Degradation

The RAC agreed with the DS's post public consultation response that metosulam is not readily (DSD) or rapidly (CLP) degradable, based on the results of the OECD 301B ready biodegradability test and the water/sediment studies. Although the primary degradation of metosulam is relatively rapid (DT_{50}=8.2 days) the observed mineralization is low (3.6 % AR 120days) and since the bound residues accounted for 58.7 to 67.5%, there was not enough evidence to show that all major degradation products are non-classifiable for the environment. Metosulam is hydrolytically stable.

Bioaccumulation

Based on a measured log Kow value of 0.2 (pH7 and 20 °C) metosulam is not likely to bioaccumulate. In a 96-hour BCF test, there was no evidence of bioaccumulation of metosulam in fish tissues after exposure to concentrations of 0.08 mg/l (the measured values were < 20% lower than the nominal) and 0.8 mg/l (the measured values were below 10% to 25% of the nominal). The BCF could not be calculated as the radioactivity levels in fish were below the limit of quantification. Then it is assumed that the BCF is below the cut-off values of the bioaccumulation criteria 500 (CLP) and 100 (DSD). RAC considers metosulam as not bioaccumulative.

Aquatic toxicity
There is information on short-term and long-term toxicity to fish, aquatic invertebrates, algae, and aquatic plant *Lemna minor*, the latter beingquite clearly the most sensitive species with a 7-day ErC_{50} value of 0.789 µg/l for *L. minor* are: and a 7-day NOErC of 0.15 µg/l. These values are based on time weighted average concentrations And the RAC considers that these can be used for classification although effect concentration based on geometric mean concentrations would have been preferred. Given the high level of losses of the test substance, the expression of Lemna results as time-weighted averages might be a more conservative indication of toxicity.

Conclusion on classification
Acute
Metosulam is not rapidly degradable, is non bioaccumulating, and the lowest acute toxicity value is in the range $0.0001 < L(E)C_{50} \leq 0.001$ mg/l.
Chronic
Metosulam is not rapidly degradable, and the lowest chronic toxicity value is in the range $0.0001 < NOEC \leq 0.001$ mg/l.

RAC concludes that metosulam fulfils the CLP criteria for classification as Aquatic Acute 1, M= 1000 and Aquatic Chronic 1, M=100 (N, R50-53 with specific concentration limits C≥0.025% N; R50-53; 0.0025%≤C<0.025% N; R51-53; 0.00025%≤C<0.0025% R52-53 according to DSD).

References:

Timchalk, C.; Dryzga M. D.; Johnson, K. A.; Eddy, S. L.; Freshour, N. L.; Nolan, 1992, R. J., Pharmacokinetics and metabolism of 14C-labeled XDE-511 in rat, mouse and dog, The Dow Chemical Company, Midland, MI, USA, Bayer CropScience AG, Report No.: DR-0276-3986-045, Edition Number: MO-01-020285, Date: 28.05.1992, GLP, unpublished, II A, 5.1 /02, XDE-511, also filed: II A, 6.2.1 /02

ANNEXES:

Annex 1 Background Document (BD) gives the detailed scientific grounds for the opinion. It is based on the CLH report prepared by the dossier submitter; the evaluation performed by the RAC is contained in RAC boxes.

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