Committee for Risk Assessment

RAC

Annex 1

Background document

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

1,2,4]triazolo[1,5-a]pyrimidine-2-sulfonamide, N-(2,6-dichloro-3-methylphenyl)-5,7-dimethoxy- (metosulam)

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

CLH-O-0000002525-76-03/A1

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

7 June 2013

CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2

Substance Name: METOSULAM

EC Number:	**Not allocated**
CAS Number:	139528-85-1
Index Number:	**not allocated**

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1. Substance identity: metosulam

Substance name:	**Metosulam**
EC number:	**Not allocated**
CAS number:	139528-85-1
Annex VI Index number:	**Not allocated**
Degree of purity:	**≥980 g/kg**
Impurities:	**See confidential annex**

1.2 Harmonised classification and labelling proposal

Table 2. The current Annex VI entry and the proposed harmonised classification: metosulam

	CLP Regulation	**Directive 67/548/EEC (Dangerous Substances Directive; DSD)**
Current entry in Annex VI, CLP Regulation		
Current proposal for consideration by RAC	Carc. Cat. 2 – H351 STOT-RE 2– H373 Acute category 1 – H400 Chronic category 1 – H410	Carc. Cat. 3; R40 Xn; R48/22 N; R50/53
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Carc. Cat. 2 – H351 STOT-RE 2– H373 Acute category 1 – H400 M-factor (acute): 1000 Chronic category 1 – H410 M-factor (chronic): 10	Carc. Cat. 3; R40 Xn; R48/22 N; R50/53 Specific Concentration Limits: C≥0.025% N; R50/53 0.0025%≤C<0.025% N; R51/53 0.00025%≤C<0.0025%

		R52/53

1.3 Proposed harmonised classification and labelling based on CLP Regulation and/or DSD criteria

Table 3. Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	None		None	Data conclusive but nott sufficient for classification
2.2.	Flammable gases	None		None	Not adequate
2.3.	Flammable aerosols	None		None	Not adequate
2.4.	Oxidising gases	None		None	Not adequate
2.5.	Gases under pressure	None		None	Not adequate
2.6.	Flammable liquids	None		None	Not adequate
2.7.	Flammable solids	None		None	Data conclusive but nott sufficient for classification
2.8.	Self-reactive substances and mixtures	None		None	Data lacking
2.9.	Pyrophoric liquids	None		None	Not adequate
2.10.	Pyrophoric solids	None		None	Data lacking
2.11.	Self-heating substances and mixtures	None		None	Not adequate
2.12.	Substances and mixtures which in contact with water emit flammable gases	None		None	Data lacking
2.13.	Oxidising liquids	None		None	Not adequate
2.14.	Oxidising solids	None		None	Data conclusive but not sufficient for classification
2.15.	Organic peroxides	None		None	Not adequate
2.16.	Substance and mixtures corrosive to metals	None		None	Data lacking
3.1.	Acute toxicity - oral	None		None	Data conclusive but not sufficient for classification
	Acute toxicity - dermal	None		None	Data conclusive but not sufficient for classification
	Acute toxicity - inhalation	None		None	Data conclusive but not sufficient for classification
3.2.	Skin corrosion / irritation	None		None	Data conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation	None		None	Data conclusive but not sufficient for classification
3.4.	Respiratory sensitisation	None		None	Data lacking
3.4.	Skin sensitisation	None		None	Data conclusive but not sufficient for classification
3.5.	Germ cell mutagenicity	None		None	Data conclusive but not sufficient for classification
3.6.	Carcinogenicity	Carc. Cat. 2 –			

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
		H351			
3.7.	Reproductive toxicity	None		None	Data conclusive but not sufficient for classification
3.8.	Specific target organ toxicity –single exposure	None		None	Data conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	STOT-RE. 2 – H373			
3.10.	Aspiration hazard	None		None	Not evaluated
4.1.	Hazardous to the aquatic environment	Acute category 1 – H400 Chronic category 1 – H410	1000 (acute) 10 (chronic)	None	
5.1.	Hazardous to the ozone layer				Data lacking

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Signal word: Warning
Hazard statements:
- H351: Suspected of causing cancer
- H373: May cause damage to organs (retina, kidney) through prolonged or repeated exposure
- H410: Very toxic to aquatic life with long lasting effects

Precautionary statements: not harmonized
Pictograms: GHS09, GHS08

Proposed notes assigned to an entry:

Table 4. Proposed classification according to DSD

Hazardous property	**Proposed classification**	**Proposed SCLs**	**Current classification [1)]**	**Reason for no classification [2)]**
Explosiveness	None		None	Data conclusive but not sufficient for classification
Oxidising properties	None		None	Data conclusive but not sufficient for classification
Flammability	None		None	The test substance was found to be non-flammable
Other physico-chemical properties *[Add rows when relevant]*	None		None	No other relevant physico-chemical properties, not sufficient for classification
Thermal stability	None		None	No decomposition before 190°C (with a 99.1 % purity test item)
Acute toxicity	None		None	Data conclusive but not sufficient for classification
Acute toxicity – irreversible damage after single exposure	None		None	Data conclusive but not sufficient for classification
Repeated dose toxicity	R48/22			
Irritation / Corrosion	None		None	Data conclusive but not sufficient for classification
Sensitisation	None		None	Data conclusive but not sufficient for classification
Carcinogenicity	Carc. Cat. 3; R40			
Mutagenicity – Genetic toxicity	None		None	Data conclusive but not sufficient for classification
Toxicity to reproduction – fertility	None		None	Data conclusive but not sufficient for classification
Toxicity to reproduction – development	None		None	Data conclusive but not sufficient for classification
Toxicity to reproduction – breastfed babies. Effects on or via lactation	None		None	Data conclusive but not sufficient for classification
Environment	N, R50/53	C≥0.025% N, R50-53 0.0025%≤C<0.025% N, R51-53 0.00025%≤C<0.0025 % R52-53	None	

[1)] Including SCLs
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Indication of danger: N Xn
R-phrases: R40, R48/22, R50/53
S-phrases: S2-36/37-46, S60, S61

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

No previous classification.

There is no available registration dossier at the date of November the 30th 2011.

2.2 Metosulam is not listed in the Annex I of the 67/548/EC Directive

Metosulam is not listed in the Annex I of the 67/548/EC Directive.

2.3 Short summary of the scientific justification for the CLH proposal

Oral administration of metosulam induced renal degenerative lesions in all the regulatory species tested (rat, mouse, dog and rabbit) and retinal detachment and necrosis in dogs, at low dose-levels in studies of various duration (from 2 weeks to 52 weeks-2 years). Aplication of the Haber's rule justify classifying metosulam as **STOT-RE. 2 – H373** according to the CLP Regulation**.**

Furthermore oral administration of metosulam to rats induced renal cortical adenomas and adenocarcinomas that may be due, based on mechanistic investigations, to the renal degenerative lesions occurring at the same range of doses after a short-term duration administration in this specie. The lack of genotoxicity potential and mechanistic data available, justify classifying metosulam as **Carc. Cat. 2 – H351**, according to the CLP Regulation.

Toxicity studies for algae and aquatic plants EC50s at concentrations ≤ 1 mg/L were observed. In addition, metosulam is rapidly biodegradable and it is unlikely for the substance to bioaccumulate in aquatic organisms (no evidence of bioaccumulation in fish tissues and log Kow < 3). As a consequence and according to the CLP Regulation, metosulam should be classified as Aquatic Acute 1 – Aquatic Chronic 1. Based on the toxicity data for *Lemna minor* (ErC50 = 0.000789 mg/L and NOEC = 0.00015 mg/L) M-factors of 1000 (acute) and 10 (chronic) are also proposed.

2.4 Current harmonised classification and labelling

No current harmonised classification in Annex VI of CLP.

2.4.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

None

2.4.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

None

2.5 Current self-classification and labelling

2.5.1 Current self-classification and labelling based on the CLP Regulation criteria

Not classified.

2.5.2 Current self-classification and labelling based on DSD criteria

Not classified.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Metosulam is currently not classified according to Annex VI of CLP.
Metosulam is an active substance in the meaning of Directive 91/414/EEC. In accordance with Article 36(2) of the CLP Regulation, Metosulam shall be subjected to harmonised classification and labelling.

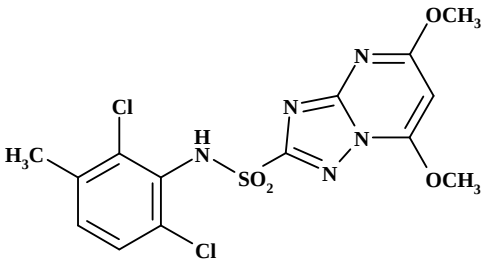
Part B.

SCIENTIFIC EVALUATION OF THE DATA

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5. Substance identity

EC number:	Not allocated
EC name:	
CAS number (EC inventory):	Not allocated
CAS number:	139528-85-1
CAS name:	[1,2,4]Triazolo[1,5-*a*]pyrimidine-2-sulfonamide, *N*-(2,6-dichloro-3-methylphenyl)-5,7-dimethoxy-
IUPAC name:	2',6'-dichloro-5,7-dimethoxy[1,2,4]triazolo[1,5-*a*]pyrimidine-2-sulfon-*m*-toluidide Fr : as included in the EFSA Journal 2010; 8(5):1592, the IUPAC name is "2′,6′-dichloro-5,7-dimethoxy-3′-methyl[1,2,4]triazolo[1,5-a]pyrimidine-2-sulfonanilide. This name has been checked by EFSA
CLP Annex VI Index number:	Not allocated
Molecular formula:	$C_{14}H_{13}Cl_2N_5O_4S$
Molecular weight range:	418.26
Structural formula:	

1.2 Composition of the substance

Table 6. Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Metosulam		≥ 980 g/kg	
Impurities	See confidential annex	See confidential annex	

Current Annex VI entry:

No harmonised classification

Table 7. Impurities (non-confidential information)

Impurities are confidential. See confidential annex.

Table 8. Additives (non-confidential information)

None

1.2.1 Composition of test material

purity of the test material for the PC studies given in table 9

1.3 Physico-chemical properties

Table 9. Summary of physico - chemical properties of Metosulam

Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
State of the substance	Cream-coloured powder (purity 99.1%)	Kraft, N. M., Swayze K. M., 1991c	Observation
Melting/freezing point	No melting point before decomposition (190°C with a 99.1 % purity test item)	Smeykal, H., 2008	Measured
Boiling point	No boiling point before decomposition (190°C with a 99.1 % purity test item)	Smeykal, H 2008	Measured
Relative density	Relative Density is of 1.49 at 20°C using a pycnometer method and a 99.1 % purity test item	Swayze, K. M., Kraft, N. M., 1991b	Measured
Vapour pressure	$1*10^{-12}$ Pa (at 25°C, 99.1 % purity test item)	Swayze, K. M. 1992	Extrapolated (measurements from 83 to 94°C)
Surface tension	69.6 mN/m (20°C, 96 %) at 202 mg/L 71.6 mN/m (20°C, 96%) at 101 mg/L	Knowles, 1992	measured
Water solubility	using the shake flask method. 0.2 g/L (20°C, 99.1 %, in distillate water un buffered) 0.1 g/L (20°C, 99.1 %, pH 5) 0.7 g/L (20°C, 99.1 %, pH 7) 5.6 g/L (20°C, 99.1 %,pH 9)	Swayze K. M., Kraft, N. M., 1991, included in Swayze, K. M. 1992 supplemented by Swayze, K. M., 1994a,	Measured
Partition coefficient n-octanol/water	At 20°C , purity 99.3% using the shake flask method: pH 4 : Log P_{OW} = 1.8 pH 7 : Log P_{OW} = 0.2 pH 9 : Log P_{OW} = -1.1	Bogdoll, B, 2008	Measured
Flash point	Not relevant. Metosulam is a solid	-	-
Flammability	Not flammable (using a 96% purity test item)	Knowles, S. J. , 1991	Measured
Explosive properties	No explosive properties (using a 96% purity test item)	Knowles, 1991	Measured
Self-ignition temperature	Metosulam is not auto inflammable up to 400°C using a 96 % purity test item	Smeykal, 2008	Measured
Oxidising properties	No oxidizing properties using a 96% purity test item	Knowles, 1992	Measured
Granulometry	Data missing	-	
Stability in organic solvents and identity of relevant degradation products	No data		
Dissociation constant	Pka = 5.5 (using a 99.3 % purity test item)	Wiche and Bogdoll, 2007	Measured
Viscosity	Not relevant, metosulam is a solid	-	-
Henry's law constant	$8*10^{-13}$ $Pa.m^3.mol^{-1}$ at 20 °C	Watson, P. A. 1992	Calculated from vapour pressure and solubility in

			water
Solubility in organic solvent	At 20°C (purity 99.1%) : acetonitrile : 10 g/L methanol : 1.9 g/L 1-octanol : 0.2 g/L n-hexane : < 0.2 g/L toluene : < 0.2 g/L methylene chloride : 6.0 g/L acetone : 7.8 g/L Ethyl acetate: 1.0 g/L	Swayze K. M., Kraft, N. M., 1991, included in Swayze, K. M. 1992 Swayze, K. M, 1994a	Measured
UV/VIS absorption (max.) incl. ε ‡ (state purity, pH)	$\varepsilon = 5.5 \times 10^4$ L.mol^{-1}.cm^{-1}at 206 nm at pH 4 $\varepsilon = 6.7 \times 10^4$ L.mol^{-1}.cm^{-1}at 212 nm at pH 7 $\varepsilon = 7.1 \times 10^4$ L.mol^{-1}.cm^{-1}at 212 nm at pH 9	Hellpointner, E., 2002	Measured at pH 4, 7, and 9 and in pure water
Storage stability at 25°C and 5°C	No data		

2 MANUFACTURE AND USES

2.1 Manufacture

Not relevant in this dossier

2.2 Identified uses

Metosulam is an herbicide intended to be used in potatoes, wheat, apples/pears and peaches.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 10. Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference
EEC A10 (flammability)	negative		Knowles, S. J. , 1991
EEC A 16 (auto-flammability)	negative		Knowles, S. J. , 1991
EEC A14 (explosivity)	negative		Knowles, S. J. , 1991
EEC A 17 (oxidizing properties)	negative		Knowles, S. J. , 1992

3.1 Explosive properties

Metosulam is a stable organic substance. None of these components or grouping are associated with explosive hazards. All are stable groupings in high oxidation states. In the Knowles, 1991 (ref MO-01-006757), a Koenen test apparatus was used for the determination of heat sensitivity, a fall hammer for sensitivity to shocks, and a friction test apparatus for sensitivity to friction. All tests were negative.

3.2 Flammability

Metosulam is an organic compound. In the study from Knowles, S. J. , 1991, metosulam has been shown not to propagate the flame. In the same study, no ignition was detected below 400°C.

The determination of flash point is not relevant because the active substance is a solid and as there is no melting point of metosulam below 40°C

So we can conclude that Metosulam is not flammable.

3.3 Oxidising potential

Oxidising compounds are materials that can easily transfer oxygen to other compounds i.e. they contain weakly bound oxygen, for example NO_3 and peroxides. Bound oxygen must also become available through a low energy degradation route with a low energy of activation. The oxygens in metosulam are including in OCH_3 or SO_2 groups .link to strong energetic resonance groups. The decomposition temperature of metosulam is high (190°C) indicating a high energy of activation. In the Knowles' study (1992) metosulam was shown not to have oxidising properties.

3.3.1 Comparison with criteria

-

3.3.2 Conclusions on classification and labelling

-

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information (refer to the DAR on metosulam)

After oral administration of metosulam to rats, dogs or mice, the absorption is quick with Tmax reached between 4-6 hours after dosing. The extent of absorption is species-dependent: it is high in rats (59-90% of the dose) but much lower in mice (21%) and dogs (19%). In rats it is sex-dependent (59-76% in males vs. 71-90% in females), enhanced after repeated dosing but characterized by a moderate saturation at higher dose-levels.

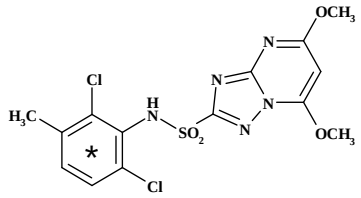
Following single [^{14}C]-metosulam oral administration to rats or dogs, radioactivity is widely distributed throughout the body. Total tissue residue concentrations are maximal in the plasma. At all times, the ratios of tissue/plasma levels remain lower than 1, demonstrating the lack of potential tissular accumulation of metosulam.

Metosulam is mainly metabolized by aliphatic oxidation of the aniline methyl group to form M05 (3-OH metosulam) and O-demethylation of the triazolpyrimidine 5-methoxy group to produce M03 (5-OH-metosulam). Oxidation of the aniline ring occurs in rats to produce M07 (4-OH-metosulam), which is secondarily conjugated either with sulphate to form M11 (4-OH-metosulam sulphate) or with glucose to form M08 (4-OH metosulam glucoside). In goats, M01 (ATSA) was identified in feces, but not in any tissue or in milk. The extent of metabolism is extensive in mice, moderate in rats and minimal in dogs and goats.

Excretion routes and rates were sex-dependent in rats. Most of the excretion occurred within 48h in females (79 to 85% of the administered dose), while it was slightly slower in males, with only 48 to 60% of the dose excreted within 48 hours. Approximately equal amounts of radioactivity in the urine (30 – 34% of the administered dose) and feces (39-53% of the administered dose) were found in males, while in females, the main route of excretion was the urine (62 – 73% of the administered dose), with minor amounts (16 – 30% of the administered dose) excreted in the feces. After oral administration to dogs or mice, most of the radioactivity was excreted via the feces as non absorbed material. Approximately 40% of the total intravenously administered dose was excreted in urine in dogs, and half of this amount was excreted within 24 hours. In goats, radioactivity was rapidly and extensively eliminated mainly in the feces (54%) and urine (21.0%), with a plateau achieved on the first dosing day. Radioactive residues in the milk remained low (0.05% of the total ingested dose), and peaked at 0.027 mg equiv./kg on day 4.

The proposed metabolic schema of ^{14}C-metosulam after oral administration in rats, mice, dogs and goats is:

Figure 4.1.1-1: Proposed Metabolic Pathways for metosulam in rats, mice, dogs and goats



Metosulam

N dealkylation

Goats

feces

Methyl hydroxylation

Rats

Mice

Aniline hydroxylation

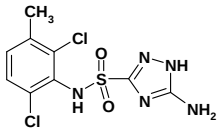
Dogs

Rats

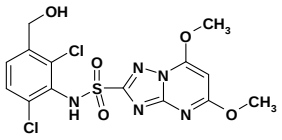
ring O-dealkylation

Rats

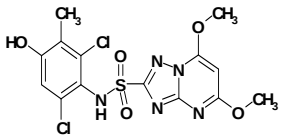
Mice



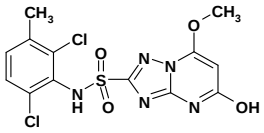
M01 (ATSA)



M05 (3-OH metosulam)



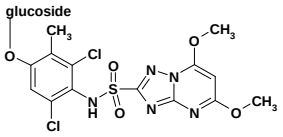
M07 (4-OH-metosulam)



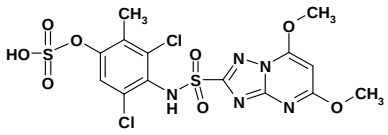
M03 (5-OH metosulam)

glucosidation

Sulfate conjugation



M08 (4-OH-metosulam glucoside)



M11 (4-OH-metosulam sulfate)

4.1.2 Human information

No information on toxicokinetics of metosulam in humans is available.

4.1.3 Summary and discussion on toxicokinetics

After oral administration to rats, dogs or mice, metosulam is rapidly absorbed and widely distributed throughout the body without tissular accumulation. The extent of absorption is species-dependent (much higher in rats) and sex-dependent in rats (higher in females).

Excretion routes and rates were sex-dependent in rats (slower in males). Metosulam is approximately equally excreted in the urine and feces in males, while in females, the main route of excretion was the urine. Metosulam was mainly excreted via the feces in dogs or mice.

Metosulam is mainly metabolized by aliphatic oxidation of the aniline methyl group, O-demethylation of the triazolpyrimidine 5-methoxy group and oxidation of the aniline which is secondarily conjugated either with sulphate or with glucose. The metabolism is extensive in mice, moderate in rats and minimal in dogs and goats.

4.2 Acute toxicity

Table 11. Summary table of relevant acute toxicity studies

Method	Results	Remarks	Reference
Oral, rat *Pesticide Assessment Guidelines, Subdivision F - Hazard Evaluation: Human and Domestic Animals (EPA 1984)*	LD_{50} >5000 mg/kg	-	Lockwood DD, Szabo JR (1989)
Inhalation, rat *OECD N° 403 (1981)*	LC_{50} > 1.9 mg/L	No death. Maximal attainable concentrations tested	Nitschke KD, Lomax LG, Crissman JW (1991)
Dermal, rabbit *Pesticide Assessment Guidelines, Subdivision F - Hazard Evaluation: Human and Domestic Animals (EPA 1984)*	LD_{50} >2000 mg/kg	-	Lockwood DD, Szabo JR (1989)

4.2.1 Non-human information

4.2.1.1 *Acute toxicity: oral*

No death related to a single oral administration of metosulam to Fischer rats at the dose-level of 5,000 mg/kg was observed during a subsequent 2-week observation period. Effects were limited to transient diarrhea.The acute oral LD_{50} of metosulam in rats is higher than 5,000 mg/kg bw for both sexes under the conditions of the study.

4.2.1.2 *Acute toxicity: inhalation*

During a 2-week observation period, there was no death or significant clinical signs in rats after a single 4-hour inhalation of metosulam at the maximal attainable concentration. Therefore, no further testing is required.

Taking into account the exposure with finest particle size (twice ground aerosol), the acute inhalation LC50 of metosulam in rats is higher than 1.9 mg/L under the conditions of the study.

4.2.1.3 *Acute toxicity: dermal*

No death related to a single 24-hour dermal application of metosulam to New Zealand White rabbits at the dose-level of 2,000 mg/kg was observed during a subsequent 2-week observation period. The acute dermal LD50 of metosulam in rabbits is higher than 2,000 mg/kg bw for both sexes under the conditions of the study.

4.2.1.4 *Acute toxicity: other routes*

No data available.

4.2.2 Human information

No reported incidents of adverse effects during the manufacture or formulation of metosulam.

4.2.3 Summary and discussion of acute toxicity

Metosulam is not acutely toxic if swallowed, by skin contact or after inhalation.

4.2.4 Comparison with criteria

The CLP criteria for classification of substances for acute toxicity, are as follow:
" Substances can be allocated to one of four toxicity categories based on acute toxicity by the oral, dermal or inhalation route according to the numeric criteria shown in Table 4.2.4-1. Acute toxicity values are expressed as acute toxicity estimates (ATE)."

Table 4.2.4-1: Acute toxicity hazard categories and acute toxicity estimates (ATE) defining the respective categories

Exposure route	Category 1	Category 2	Category 3	Category 4
Oral (mg/kg bodyweight)	ATE ≤ 5	5 < ATE ≤ 50	50 < ATE ≤ 300	300 < ATE ≤ 2000
Dermal (mg/kg bodyweight)	ATE ≤ 50	50 < ATE ≤ 200	200 < ATE ≤ 1000	1000 < ATE ≤ 2000

Dusts and Mists (mg/L)	ATE ≤ 0.05	0.05 < ATE ≤ 0.5	0.5 < ATE ≤ 1.0	1.0 < ATE ≤ 5.0

Considering that:
- oral and dermal LD50 are higher than 2000 mg/kg bw, and that
- there was no death in rats after inhalation of metosulam at the maximal attainable concentration,

No classification is required for metosulam under either Directive 67/548/EEC or the CLP Regulation.

4.2.5 Conclusions on classification and labelling

No classification is required for metosulam under either Directive 67/548/EEC or the CLP Regulation.

4.3 Specific target organ toxicity – single exposure (STOT SE)

No specific target organ toxicity after single exposure.

4.4 Irritation

4.4.1 Skin irritation

Table 12. Summary table of relevant skin irritation studies

Method	Results	Remarks	Reference
Skin irritation, rabbit *US EPA series 81-5 (1982)*	**Not irritant**	-	Lockwood DD (1989)

4.4.1.1 *Non-human information*

No cutaneous irritation was observed at any test site during the study (see Table 4.4.1.1-1).

Table 4.4.1.1-1: Individual and mean skin irritation scores according to the Draize scheme

Animal	ERYTHEMA SCORE						Oedema score					
No. Sex	1 M	2 M	3 M	4 F	5 F	6 F	1 M	2 M	3 M	4 F	5 F	6 F
0.5h	0	0	0	0	0	0	0	0	0	0	0	0
24 h	0	0	0	0	0	0	0	0	0	0	0	0
48 h	0	0	0	0	0	0	0	0	0	0	0	0
72 h	0	0	0	0	0	0	0	0	0	0	0	0
Irritation Index	0						0					

Under the conditions of the study, no local inflammatory reaction was observed after topical cutaneous application of metosulam to the skin of rabbits for 4 hours under occlusion.

4.4.1.2 *Human information*

No reported incidents of adverse effects during the manufacture or formulation of metosulam.

4.4.1.3 *Summary and discussion of skin irritation*

Under the conditions of the study, no local inflammatory reaction was observed after topical cutaneous application of metosulam to the skin of rabbits for 4 hours under occlusion.

4.4.1.4 *Comparison with criteria*

The CLP criteria for classification of substances for skin irritation, are as follow :

- at least 2 of 3 tested animals have a mean score of ≥ 2,3 - ≤ 4,0 for erythema/eschar or for oedema from gradings at 24, 48 and 72 hours after patch removal or, if reactions are delayed, from grades on 3 consecutive days after the onset of skin reactions; or
- inflammation that persists to the end of the observation period normally 14 days in at least 2 animals, particularly taking into account alopecia (limited area), hyperkeratosis, hyperplasia, and scaling; or
- in some cases where there is pronounced variability of response among animals, with very definite positive effects related to chemical exposure in a single animal but less than the criteria above.

Considering that no cutaneous irritation was observed at any test site during the study, no classification is required for metosulam under either Directive 67/548/EEC or the CLP Regulation.

4.4.1.5 *Conclusions on classification and labelling*

No classification is required for metosulam under either Directive 67/548/EEC or the CLP Regulation.

4.4.2 Eye irritation

Table 13. Summary table of relevant eye irritation studies

Method	Results	Remarks	Reference
Eye irritation, rabbit *US EPA series 81-4 (1982)*	**Not irritant**	-	Lockwood DD (1989)

4.4.2.1 *Non-human information*

All local observations are reported in Table 4.4.2.1-1. There were limited to slight congestion of the conjunctival blood vessels in 3/6 animals (sex not mentioned) and had disappeared 24 hours after instillation.

The untreated eyes of all rabbits were overtly normal at all examinations.

Table 4.4.2.1-1: Eye irritation score in rabbits after a single application of metosulam at 0.1g into the conjunctival sac

Clinical signs	**Animal**					
	1	**2**	**3**	**4**	**5**	**6**
Conjunctival redness						
After 1 hr	1	0	1	0	0	1
After 24 hr	0	0	0	0	0	0
After 48 hr	0	0	0	0	0	0
After 72 hr	0	0	0	0	0	0
Mean score 24-72 hr	0	0	0	0	0	0
Group mean score 24-72 hr	**0**					
Conjunctival chemosis						
After 1 hr	0	0	0	0	0	0
After 24 hr	0	0	0	0	0	0
After 48 hr	0	0	0	0	0	0
After 72 hr	0	0	0	0	0	0
Mean score 24-72 hr	0	0	0	0	0	0
Group mean score 24-72 hr	**0**					
Conjunctival discharge						
After 1 hr	0	0	0	0	0	0
After 24 hr	0	0	0	0	0	0
After 48 hr	0	0	0	0	0	0
After 72 hr	0	0	0	0	0	0
Mean score 24-72 hr	0	0	0	0	0	0
Group mean score 24-72 hr	**0**					
Cornea opacity						
After 1 hr	0	0	0	0	0	0
After 24 hr	0	0	0	0	0	0
After 48 hr	0	0	0	0	0	0
After 72 hr	0	0	0	0	0	0
Mean score 24-72 hr	0	0	0	0	0	0
Group mean score 24-72 hr	**0**					
Iridial inflammation						

After 1 hr	0	0	0	0	0	0
After 24 hr	0	0	0	0	0	0
After 48 hr	0	0	0	0	0	0
After 72 hr	0	0	0	0	0	0
Mean score 24-72 hr	0	0	0	0	0	0
Group mean score 24-72 hr	**0**					

Under the conditions of the study, application of metosulam to the eye of New Zealand White rabbits at the dose-level of 0.1 g only induced minimal conjunctival redness during the first 24 hours after application.

4.4.2.2 *Human information*

No reported incidents of adverse effects during the manufacture or formulation of metosulam.

4.4.2.3 *Summary and discussion of eye irritation*

Under the conditions of the study, application of metosulam to the eye of New Zealand White rabbits at the dose-level of 0.1 g only induced minimal conjunctival redness during the first 24 hours after application.

4.4.2.4 *Comparison with criteria*

The CLP criteria for classification of substances for eye irritation, are as follow :

- Category 1 (Irreversible effects on the eye) :
  - at least in one animal effects on the cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days; and/or
  - at least in 2 of 3 tested animals, with a positive response of corneal opacity ≥ 3 and/or iritis > 1.5
- Category 2 (Irritating to eyes) :
  - at least in 2 of 3 tested animals, with a positive response of corneal opacity ≥ 1 and/or iritis ≥ 1, and/or conjunctival redness ≥ 2 and/or conjunctival oedema (chemosis) ≥ 2 calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material, and which fully reverses within an observation period of 21days.

Considering that there were limited to slight congestion of the conjunctival blood vessels that disappeared 24 hours after instillation, no classification is required for metosulam under either Directive 67/548/EEC or the CLP Regulation.

4.4.2.5 *Conclusions on classification and labelling*

No classification is required for metosulam under either Directive 67/548/EEC or the CLP Regulation.

4.4.3 Respiratory tract irritation

No non-human data available and no reported incidents of adverse effects during the manufacture or formulation of metosulam.

4.5 Corrosivity

4.5.1 Non-human information

No non-human data available and no reported incidents of adverse effects during the manufacture or formulation of metosulam.

Metosulam is not considered as a corrosive substance (refer to non-human irritation data).

4.6 Sensitisation

Table 14. Summary table of relevant skin sensitizationstudies

Method	Results	Remarks	Reference
Skin sensitisation, guinea pig - Magnusson and Kligman maximization method *OECD N° 406 (1981)*	Not sensitizing	No positive control was included in the study as required by EC guideline B6 (92/69/CEE)	Johnson IR (1993)
Skin sensitisation, guinea pig - Modified Buehler method *US EPA series 81-6 (1982)*	Not sensitizing	No negative control. The positive control used in the study is not listed in EC guideline B6 (92/69/CEE)	Lockwood DD (1989)

4.6.1 Skin sensitisation

4.6.1.1 *Non-human information*

Two studies investigating the potential skin sensitization properties of metosulam in guinea pigs were conducted:

- one under the maximization protocol of Magnusson and Kligman (with batch RMM 1940, purity 97.8%),

No significant responses (slight erythema or a more marked reaction) were observed in the test or control animals following challenge application of purified water alone, 5% or 30% metosulam metosulam technical in purified water.

Table 4.6.1.1-1: Dermal responses to challenge test for each group.

Group		1 (control)			2 (test)		
Topical treatment		PW	5% metosulam in PW	30% metosulam in PW	PW	5% metosulam in PW	30% metosulam in PW
24 h examination	Incidence	0	0	0	0	0	0
	Severity	0	0	0	0	0	0
48 h examination	Incidence	0	0	0	0	0	0
	Severity	0	0	0	0	0	0

PW: purified water

- a second one without maximization (Buehler test, with batch AGR 265439, purity 98.8%).

No edema was observed in any animal. Erythema was observed in none of the test-item treated animals, but it was observed in 8/10 positive controls both at 24h and 48h post-challenge.

Table 4.6.1.1-2: Dermal responses (erythema[(a)]) to challenge test for each group.

Topical treatment		Metosulam	D.E.R*331
24 h examination	Incidence	0	8
	Severity	0	1.625
48 h examination	Incidence	0	8
	Severity	0	1.75

(a): no edema was observed at any time in any animal

In conclusion, using both the Magnusson-Kligman maximization test and the Buehler test, metosulam has no potential to induce delayed hypersensitivity in male guinea-pigs.

4.6.1.2 *Human information*

No reported incidents of adverse effects during the manufacture or formulation of metosulam.

4.6.1.3 *Summary and discussion of skin sensitisation*

In conclusion, using both the Magnusson-Kligman maximization test and the Buehler test, metosulam has no potential to induce delayed hypersensitivity in male guinea-pigs.

4.6.1.4 *Comparison with criteria*

The CLP criteria for classification of substances for skin sensitization, are as follow:
" Substances shall be classified as skin sensitisers (Category 1) in accordance with the following criteria:

- if there is evidence in humans that the substance can lead to sensitisation by skin contact in a substantial number of persons, or
- if there are positive results from an appropriate animal test.
    - Guinea pig maximisation test (GPMT) Redness in ≥ 30% of the test animals
    - Buehler occluded patch test Redness in ≥ 15% of the test animals.

Considering that no specific effect is reported in human, and that negatives results were obtained from the two animal tests, no classification is required for metosulam under either Directive 67/548/EEC or the CLP Regulation.

4.6.1.5 *Conclusions on classification and labelling*

No classification is required for metosulam under either Directive 67/548/EEC or the CLP Regulation.

4.6.2 Respiratory sensitisation

No non-human data available and no reported incidents of adverse effects during the manufacture or formulation of metosulam.

4.7 Repeated dose toxicity

Table 15. Summary table of relevant repeated dose toxicity studies

Method	Results	Remarks	Reference
Oral, 2 weeks, Beagle dogs 25, 100, 250, 500, 1,000 mg/kg/d (dietary administration, 2 weeks) 2000 mg/kg/d (gelatin capsules, 5 days)	Oral administration of metosulam in gelatin capsules: no toxic effect in male Beagle dogs Dietary administration of metosulam **≥ 100 mg/kg bw/d:** - lower food intake with weight loss - retinal detachment with necrosis, - renal degenerative lesions. **≥ 250 mg/kg bw/d:** - mortality - firm lungs. **≥ 500 mg/kg bw/d:** - weakness - presence of dark ingesta in the digestive lumen. **At 1000 mg/kg bw/d only:** - hemorrhage of digestive lumen, eyes, stomach or lymph nodes.	Range-finding study	Szabo JR, Rachunek BL (1989)
Oral (diet), 2 weeks, CD-1 mice 100, 500, 1000, 2000, 5000 mg/kg/d	**≥ 2000 mg/kg bw/day:** Liver lesions: centrolobular hypertrophy in males, **At 5000 mg/kg bw/day only:** decreased liver vacuolation	Range-finding study	Szabo JR, Davis NL (1988)
Oral (diet), 2 weeks, SD rats 100, 500, 1000, 2000, 5000 mg/kg/d	**≥ 100 mg/kg bw/day**: Lower body weights, associated with a decrease in diet palatability	Range-finding study	Szabo JR, Davis NL (1988)
Oral (diet), 2 weeks, LE rats 1000, 5000 mg/kg/d	No relevant effect.	Range-finding study	Grandjean M, Szabo JR (1989)
Oral (gavage), 2 weeks, NZW female rabbits 300, 600, 1000 mg/kg/d	**≥ 300 mg/kg bw/day**: - higher mortality, preceded by soiling and anorexia, and subsequently lower body weight gains, - renal tubular epithelial cell degeneration and necrosis. **≥ 600 mg/kg bw/day**: - higher kidney weights - focal necrosis and inflammation of the gallbladder.	Range-finding study	Yano BL, Breslin WJ, Liberacki AB (1990)
Oral (diet), 13 weeks, Beagle dogs 5, 25, 50 mg/kg/d *Pesticide Assessment Guidelines, Subdivision F (EPA 1982). Japan MAFF (1985).*	**≥ 25 mg/kg bw/d:** - lower CK ; - renal collecting duct degeneration. **At 50 mg/kg bw/d only:** - retinal detachment and atrophy - lower platelet counts - lower potassium levels, higher PAL - lower urine specific gravity - periportal aggregates of mononuclear cells in liver from females.		Szabo JR, Davis NL (1990)
Oral (diet), 13 weeks, CD-1 mice 250, 1000, 2000 mg/kg/d *Pesticide Assessment Guidelines, Subdivision F (EPA 1982). Japan MAFF (1985).*	**≥ 1000 mg/kg bw/d:** - liver hypertrophy, focal necrosis and inflammation of the liver. **At 2000 mg/kg bw/d only:** - lower platelet count - renal tubule degeneration/regeneration		Szabo JR, Rachunek BL (1989)

Method	Results	Remarks	Reference
Oral (diet), 13 weeks, SD rats 10, 100, 500, 1000 mg/kg/d *Pesticide Assessment Guidelines, Subdivision F (EPA 1982). Japan MAFF (1985).*	**≥ 100 mg/kg bw/d:** - lower food intake with impact on body weight, biochemistry, mesentery fat - renal epithelium: nuclear pleomorphism. **≥ 500 mg/kg bw/d:** - lower urine specific gravity - proximal renal tubule lesions, including epithelial hypertrophy, increased basophilia. **At 1000 mg/kg bw/d only:** - minor clinical signs - lower kidney weights - decreased amount of mesentery fat in males.		Szabo JR, Grandjean M (1989)
Oral (diet), 52 weeks, Beagle dogs, 3, 10, 37.5 mg/kg/d *OECD N° 452 (1984)*	**At 37.5 mg/kg/d:** - retinal degeneration and detachment - lower platelet counts - lower CK, higher PAL and creatinine in both sexes, and lower potassium in females - gallbladder: mucin accumulation - kidneys: mononuclear aggregates, pyelitis, urocystitis, epithelial degeneration		Barna-Lloyd T, Szabo JR, Rachunek BL (1992)
Dermal, 3 weeks (15 applications), NZW rabbits 100, 500, 1000 mg/kg/d *OECD N° 404 and 410 (1981)*	No relevant effect.		Crissman JW, Zablotny CL, Breslin WJ (1990)
Ocular, 14 days, Beagle dogs 0.1g/d	Focal conjunctival necrosis (1 dog, irritative)	Supportive	Szabo JR, Davis NL (1991)
Oral (gavage), 6-week, Cynomolgus monkeys 100 mg/kg/d	No relevant effects on eyes. Pale feces and diarrhoea.	Supportive	Makin A, Lanham DF, Gregson RL, Offer JM, Gopinath C (1990)

Method	Results	Remarks	Reference
Oral, 7-day, Sprague-Dawley rats 100 mg/kg/d	Renal tubular necrosis and degeneration/regeneration of tubules (very slight to slight)	Supportive. Preliminary evaluation of the temporal development of nephrotoxicity in male Sprague-Dawley rats. No control group (with no substance administrated)	Yano BL, Haut KT (1992)
Oral, 2-week, Sprague-Dawley rats 5, 30, 100 mg/kg/d	<u>At 30 mg/kg/d and100 mg/kg/day :</u> Renal toxicity - tubular epithelial cell degeneration/regeneration, - increased mitotic figures, - individual cell necrosis and - nuclear pleomorphism. - minor alterations in the urine sediment	Supportive. Evaluation of the initial kidney lesions induced by metosulam in Sprague Dawley rats	Yano BL, Haut KT (1992)
Oral, 2-week, Sprague-Dawley rats 100 mg/kg/d	Renal toxicity occurring at 100 mg/kg/d: - tubular epithelial cell necrosis, - degeneration/regeneration, increased mitoses, and - increased nuclear pleomorphism - increased BrdU incorporation into epithelial cell nuclei in regions of tubular epithelial cell toxicity	Supportive. Investigation of renal tubular cell turnover in rats, using bromodeoxyuridine as marker	Yano BL, Haut KT, Redmond JM (1992)

4.7.1 Non-human information

4.7.1.1 *Repeated dose toxicity: oral*

4.7.1.1.1 Szabo JR, Davis NL (1990): XRD-511 herbicide: 13-week dietary study in Beagle dogs

Four groups of 4 male and 4 female Beagle dogs (5 months old, bw range 6.7-9.2 kg for males, 5.4-8.1 kg for females) were administered technical metosulam (batch 265439, purity of 98.8%) in their diet, at dose-levels of 0, 5, 25 and 50 mg/kg/day (based on group mean body weight and food intake) for 13 weeks, before final necropsy. The first treatment day was noted day 1.

The animals were observed daily for clinical signs and mortalities. Additional evaluations including ophthalmology were performed weekly. Individual body weights were recorded on pre-test and then every 7 days. Weekly food consumption was determined on pre-test and then every 7 days.

Haematological (red and white blood cell parameters, and blood smears) and blood biochemistry investigations were performed on blood samples taken from all animals on pre-test, on week 6 and at the end of the study. Urinalysis was performed on all animals at necropsy.

After sacrifice, all animals were subjected to gross post-mortem external and internal examination. Selected organs (brain, pituitary, thyroid, adrenals, heart, liver, kidneys, testes/ovaries) were weighed. Histolopathologic examination was performed on selected organs (gross lesions, adrenal glands, aorta, bone, bone marrow, brain, cecum, cervical lymph node, cervix, colon, duodenum, epididymides, esophagus, eyes and optic nerve, gall bladder, heart, ileum, jejunum, kidneys, liver, lungs, mammary gland, mediastinal lymph node, mediastinal tissue, mesenteric lymph node, mesenteric tissue, ovaries, oviducts, pancreas, parathyroid glands, pituitary gland, prostate, rectum, spinal cord, salivary gland, sciatic nerve, skin and

subcutis, spleen, stomach, testes, thymus, thyroid gland, tongue, tonsil, trachea, urinary bladder, uterus and vagina) from all animals.

Results

There were no treatment-related differences in body weights or food consumption between treated and control animals.

At the end of the treatment, retinal detachment was clinically diagnosed at ophthalmologic examinations in all high-dose animals, and later confirmed at histopathology (see Table 4.7.1.1.1-2). There was no other treatment-related clinical sign.

At haematological examinations, platelet counts were lower in high-dose animals on week 6 (males: -34%, females: -25%) and at study termination (males: -42%, females: -30%) compared to respective controls.

Urinalysis performed at the end of the study showed lower urine specific gravity in both sexes, mainly at the high dose-level (see Table 4.7.1.1.1-1).

Blood biochemistry investigations showed lower CK in mid- and high-dose animals on weeks 6 and 13 (males: up to -40% and -64%, respectively; control values being unusually high in females, differences from treated females were not quantified). PAL were higher in high-dose animals compared to controls on weeks 6 and 13 (males: up to +100%, females: up to +182%). Phosphate and triglyceride levels in treated animals showed some variations from control values, but as those were not coherent when week 6 and week 13 values were compared (change in trend and/or not observed in the same sex, see Table 4.7.1.1.1-1), they were not considered to be treatment-related. Potassium levels were lower in high-dose females on week 6 (-20%) and in both sexes of the high-dose group on week 13 (males: -24%, females: -25%), compared to controls.

Table 4.7.1.1.1-1: Incidence (/4 animals per sex and group) of findings at hematology, urinalysis and blood biochemistry in metosulam treated and control dogs.

Dose-level (mg/kg/day)		0		5		25		50	
Sex		M	F	M	F	M	F	M	F
Investigations on week 6									
Haematology	Platelet count (103/cm3)	302	249	289	280	270	264	198	187
Blood biochemistry	Creatine kinase (U/l)	182	528	168	187	122	109	66	70
	Alcaline phosphatase (U/l)	90	80	67	86	90	99	119	173
	Phosphates (mg/dl)	6.7	5.4	6.2	4.6	6.9	5.1	7.5	5.7
	Triglycerides (mg/dl)	22.3	22.7	28.3	22.0	28.5	24.2	22.9	13.6
	Potassium (meq/l)	4.59	4.73	4.46	4.27	4.38	4.14	4.40	3.79
Investigations on week 13									
Haematology	Platelet count (103/cm3)	290	260	307	289	241	293	175	181
Blood biochemistry	Creatine kinase (U/l)	155	324	114	185	93	101	88	69
	Alcaline phosphatase (U/l)	60	72	50	67	77	81	120	203
	Phosphates (mg/dl)	6.6	5.8	6.2	4.9	5.9	4.9	5.6	5.3
	Triglycerides (mg/dl)	24.4	18.9	26.6	17.3	24.9	19.0	13.4	17.2
	Potassium (meq/l)	4.56	4.59	4.47	4.19	3.94	3.78	3.46	3.46
Urinalysis	Specific gravity	1.036	1.048	1.038	1.038	1.024	1.023	1.016	1.017

There were no differences in organ weights at the end of the study.

Histopathology confirmed retinal detachment, associated with retinal atrophy, in all high-dose animals. High-dose females showed a higher frequency of periportal aggregates of mononuclear cells in the liver. Degeneration of renal collecting ducts was observed in mid- and high-dose animals, with moderate or low intensity (very slight in males, slight in females) (Table 4.7.1.1.1-2).

Table 4.7.1.1.1-2: Post-mortem microscopic findings: incidence (/4 animals per sex and group) in metosulam treated and control dogs.

Dose-level (mg/kg/day)		0		5		25		50	
Sex		M	F	M	F	M	F	M	F
Eyes	Retinal detachment with atrophy	0	0	0	0	0	0	4	4
Liver	Periportal aggregates of mononuclear cells	1	0	1	0	0	1	1	3
Kidneys	Collecting ducts degeneration	0	0	0	0	3	4	4	4

Conclusion

Dietary administration of metosulam at dose-levels of 5, 25 or 50 mg/kg/day to Beagle dogs for 13 weeks induced:

- retinal detachment and atrophy, diagnosed both clinically and at histopathology in all animals treated at 50 mg/kg/day;
- lower platelet counts (roughly, -30%) in both sexes treated at 50 mg/kg/day;
- lower urine specific gravity in both sexes treated at 50 mg/kg/day;
- lower CK (25-50 mg/kg/day) and potassium levels (50 mg/kg/day), higher PAL (50 mg/kg/day), all present in both sexes;
- periportal aggregates of mononuclear cells in liver from females treated at 50/mg/kg/day, and renal collecting duct degeneration in both sexes (25-50 mg/kg/day).

4.7.1.1.2 Szabo JR, Rachunek BL (1989): XRD-511 herbicide: 13-week dietary toxicity study in CD-1 mice

Four groups of 10 male and 10 female CD1 mice (8 weeks old, bw range 28-34 g for males, 23-28 g for females) were administered technical metosulam (batch 265439, purity of 98.8%) in their diet, at dose-levels of 0, 250, 1,000 or 2,000 mg/kg/day (based on group mean body weight and food intake) for 13 weeks, before final necropsy. The first treatment day was noted day 1.

The animals were observed daily for clinical signs and mortalities. Physical abnormalities, activity and behavior were evaluated weekly. Individual body weights were recorded on days -1 and then every 7 days. Weekly food consumption was determined on pre-test and then every 7 days.

Haematology (red, white blood cells and blood smears) and blood biochemistry were performed on blood samples taken from all animals immediately before sacrifice.

After methoxyflurane sacrifice, all animals were subjected to gross *post-mortem* external and internal examination. Selected organs (brain, adrenals, heart, liver, kidneys, testes) were weighed. Histolopathologic examination was performed on selected organs (adrenal glands, aorta, bone, bone marrow, brain, cecum, cervix, coagulating glands, colon, duodenum, epididymides, esophagus, eyes, gall bladder, harderian gland, heart, ileum, jejunum, kidneys, liver, lungs, mammary gland, mediastinal lymph node, mesenteric lymph node, mediastinal tissue, mesenteric tissue, ovaries, oviducts, pancreas, parathyroid glands, peripheral nerve, pituitary gland, prostate, rectum, salivary glands, seminal vesicles, skeletal muscle, skin and subcutis, spinal cord, spleen, stomach, testes, thymus, thyroid gland, tongue, trachea, urinary bladder, uterus and vagina) from groups 1 and 4, and additionally on selected organs (gall bladder, kidneys, liver, lungs, gross lesions) from remaining groups.

Results

There were no clinical signs or mortalities at any dose-level.

There were no significant differences in mean body weight and food intake between treated and control groups.

At the high-dose level, males showed minimally lower RBC (-9%) and PCV (-11%, trend in females) than controls, but the amplitude of these changes were low and they were not considered to be biologically relevant. Platelet counts were moderately lower in females from mid- and high-dose groups (-16% and -23%, respectively; trend in males).

Table 4.7.1.1.2-1: Findings at Haematology in metosulam treated and control mice.

Dose-level (mg/kg/day)	0		250		1,000		2,000	
Sex	M	F	M	F	M	F	M	F
RBC (10^6/cm^3)	8.74	8.77	8.76	8.83	8.28	8.83	7.97*	8.82
PCV (%)	41.2	41.2	41.4	40.6	38.1	40.8	36.8*	39
Platelets (10^3/cm^3)	1045	911.6	966	875.1	1089	765.6*	840	705.4*

*: statistical difference, $p<0.05$

There were no treatment-related findings for any blood biochemistry parameter or at organ weight analysis.

Multifocal tubule degeneration and regeneration were observed at slightly higher frequency in kidneys from high-dose males compared to controls. Renal lesions in other groups including controls were focal in nature. Mid- and high-dose males also showed almost systematic liver hypertrophy, with dose-related extension (centrolobular: 7/9 and 1/10; panlobular: 0/9 and 2/10, for respective dose-levels of 1,000 and 2,000 mg/kg/day). This hypertrophy was also observed in half of high-dose females, but was always centrolobular. Additionally, livers from some high-dose males (2/10) showed focal necrosis and inflammation.

Table 4.7.1.1.2-2: *Post-mortem* microscopic findings: incidence (/10 animals per sex and group) in metosulam treated and control mice.

Dose-level (mg/kg/day)		0		250		1,000		2,000	
Sex		M	F	M	F	M	F	M	F
Kidneys: tubules	Degeneration/regeneration	3	3	1	2	4	2	5	2
Liver: lobules	Hypertrophy	0	0	0	0	9	0	10	5
	Focal necrosis and inflammation	0	1	0	1	0	0	2	0

Conclusion

Dietary administration of metosulam at dose-levels of 250, 1000 or 2,000 mg/kg/day to CD1 mice for 13 weeks induced:

- lower platelet count (-23%) in females treated at 2,000 mg/kg/day, this being present as a trend in males.
- histopathological lesions mainly in males: slightly higher incidence of renal tubule degeneration/regeneration (2,000 mg/kg/day), almost systematic liver lobular hypertrophy with dose-related extension (1,000-2,000 mg/kg/day, present in 5/10 high-dose females) and some rare (2/10 high-dose males) focal necrosis and inflammation of the liver.

4.7.1.1.3 Szabo JR, Grandjean M (1989): XRD-511 herbicide: 13-week dietary toxicity study in Sprague-Dawley rats

Five principal groups (numbered 1 to 5 respectively) of 10 male and 10 female Sprague-Dawley rats (8 weeks old, bw range 218-258 g for males, 146-194 g for females) were administered technical metosulam (batch 265439, purity of 98.8%) in their diet, at dose-levels of 0, 10, 100, 500 or 1,000 mg/kg/day (based on group mean body weight and food intake) for 13 weeks, before final necropsy. The first treatment day was

noted day 1. Two additional satellite groups each made of 10 males and females were treated at 0 or 1,000 mg/kg/day for 13 weeks and maintained for a 4-week recovery period, before final necropsy. All investigations were performed both in principal and satellite groups.

The animals were observed daily for clinical signs and mortalities. Physical abnormalities, activity and behavior were evaluated weekly. A functional observation battery was performed on all rats on pre-test and on days 33, 55 and 90. Individual body weights were recorded on days -1 and then every 7 days. Weekly food consumption was determined on pre-test and then every 7 days.

Haematological (red, white blood cell parameters and blood smears) and blood biochemistry investigations were performed on blood samples taken from all animals immediately before sacrifice. Urinalysis was performed on principal animals at the end of the treatment and on satellites at the end of the recovery period.

After methoxyflurane sacrifice, all animals were subjected to gross *post-mortem* external and internal examination. Selected organs (brain, adrenals, heart, liver, kidneys, testes, ovaries) were weighed. Histolopathologic examination was performed on selected organs (adrenal glands, aorta, bone, bone marrow, brain, cecum, cervix, coagulating glands, colon, duodenum, epididymides, esophagus, eyes, harderian glands, heart, ileum, jejunum, kidneys, larynx, liver, lungs, mammary gland, mediastinal lymph node, mediastinal tissue, mesenteric lymph node, mesenteric tissue, nasal tissue, ovaries, oviducts, pancreas, parathyroid glands, peripheral nerve, pituitary gland, prostate, rectum, salivary glands, seminal vesicles, skeletal muscle, skin, spinal cord, spleen, stomach, testes, thymus, thyroid gland, tongue, trachea, urinary bladder, uterus, vagina) from principal groups 1 and 5, and additionally on selected organs (adrenal glands, kidneys, liver, lungs, testes and gross lesions) from remaining pricnipal groups.

Results

There were no mortalities at any dose-level.

There was no finding at pre-test functional observation battery. On day 33, several high-dose females showed hindquarter hair loss and/or urine staining of the perineum. On day 55, a higher number of females showed these findings and fecal staining was additionally observed in some males and females from the high-dose group. On day 90, the incidence of these findings had decreased in females, and high-dose males presented urine staining but no fecal staining.

Table 4.7.1.1.3-1: Functional observation battery: maximal incidence (and corresponding day of observation) of selected clinical signs.

Nominal dose-level (mg/kg/day)	0		10		100		500		1,000	
Sex number	M 20	F 20	M 10	F 10	M 10	F 10	M 10	F 10	M 20	F 20
hindquarter hair loss	0	0	0	0	0	0	0	1 (55)	0	6 (55)
fecal staining	0	0	0	0	1 (55)	0	0	0	3 (55)	3 (55)
urine staining	0	0	0	0	0	0	0	0	2 (90)	4 (55)

In each sex, pre-treatment mean body weights were similar between groups. From day 7 until the end of the treatment, mean body weights of males treated at 100-1,000 mg/kg/day and females treated at 500-1,000 mg/kg/day were significantly and dose-relatedly lower than those of controls (from -6% to -20% when all variations are considered). During the recovery period, body weight gains remained lower in treated animals, and terminal mean body weights were still significantly lower in treated animals (up to -12%).

These findings were well correlated with food consumption: the latter was slightly (not significantly) lower in the males treated at 100-1,000 mg/kg/day and females treated at 500-1,000 mg/kg/day, with dose-relationship, from the start of the treatment almost until the end of the treatment. During the recovery, food intake in treated animals stayed lower than in controls. All these observations point to unpalatability of the diets containing the test item (differences were apparent right from the start of the treatment) but also to later onset of systemic effects of the test item (the recovery period not enabling recovery of appetite).

Table 4.7.1.1.3-2: Mean body weights (g) in metosulam treated and control rats.

Nominal dose-level (mg/kg/day)		0		10		100		500		1,000	
Sex		M	F	M	F	M	F	M	F	M	F
Treatment[p,s]	Day -1	236.9	165.6	238.8	164.5	236.8	167.0	235.4	162.9	234.8	170.3
	Day 7	295.5	192.8	289.5	192.6	277.7*	188.0	250.2*	170.2*	241.8*	171.9*
	Day 56	481.0	265.7	463.7	265.2	441.3	264.5	405.1*	223.6*	389.1*	225.1*
	Day 91	551.6	303.5	517.7	304.8	496.6*	298.8	461.5*	255.3*	445*	253.3*
Recovery[p]	Day 119	568.7	342.7	-	-	-	-	-	-	511.2*	301.8*

*: statistical difference, $p<0.05$; p: principal animals, s: satellite animals.

Once calculated using body weight and food intake data (Table 6.3.2.2.-3), the achieved dose-levels are markedly different from nominal dose-levels, and not coherent when sexes are compared. This is not coherent with the stated method of preparation of the dietary mixes, namely calculation of dietary concentrations using body weight and food intake data.

Table 4.7.1.1.3-3: Calculation of the achieved dose-level (mg/kg/day), using measured dietary concentrations (µg/g at the end of the week), mean body weight (g at the end of the week, see previous table) and mean weekly food intake (g/day).

Nominal dose-level (mg/kg/day		**10**		**100**		**500**		**1000**	
Sex		**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Days 1 to 7	Body weight (g)	289.5	192.6	277.7	188.0	250.2	170.2	241.8	171.9
	Dietary concentration (µg/g)	111	95	1260	1160	6450	5730	12900	11800
	Food intake (g/day)	23.8	17.3	21.1	15.2	16.5	12.2	16.1	12.4
	Achieved dose-level (mg/kg/day)	9.1	8.5	95.7	93.8	425.4	410.7	859.0	851.2
Days 50 to 57	Body weight (g))	463.7	265.2	441.3	264.5	405.1	223.6	389.1	225.1
	Dietary concentration (µg/g)	205	165	2080	1380	9330	7870	18000	14600
	Food intake (g/day	25.5	19.6	24.3	19.4	23.2	16.7	23.3	17.2
	Achieved dose-level (mg/kg/day)	11.3	12.2	114.5	101.2	534.3	587.8	1078.0	1115.6
Days 85 to 92	Body weight (g)	517.7	304.8	496.6	298.8	461.5	255.3	445.0	253.3
	Dietary concentration (µg/g)	189	139	1990	1510	9520	7160	18200	13000
	Food intake (g/day)	25.8	21.2	24.7	21.2	23.9	18.7	24.0	20.0
	Achieved dose-level (mg/kg/day)	9.4	9.7	99.0	107.1	493.0	524.5	981.6	1026.5

At haematological investigation, at the end of the treatment, minimally lower PCV was observed in females (up to -5%) but this was not considered to be biologically significant. After the recovery period, minimally, not biologically relevant lower RBC (-2%) was observed in males. There were therefore no treatment-related findings at haematology.

At the end of the treatment, urinalysis in males treated at 500-1,000 mg/kg/day showed minimally lower specific urine gravity (Table 4.7.1.1.3-4). There was also a slight trend towards an increase in the frequency of amorphous sediment in high-dose males and females. Although of limited biological relevance, these clinical findings were considered to be associated with the renal lesions described below. No finding was observed at the end of the recovery period.

4.7.1.1.3-4: Findings at urinalysis at the end of the treatment in metosulam treated and control rats.

Dose-level (mg/kg/day)	0		10		100		500		1,000	
Sex	M	F	M	F	M	F	M	F	M	F
Specific gravity	1.070	1.072	1.059	1.073	1.062	1.070	**1.042***	1.043	**1.033***	1.041
Amorphous sediment (semi-quantification)	0	2	0	3	0	2	1	2	1	4

*: statistical difference, $p<0.05$

The results of blood biochemistry investigations at the end of the treatment period are summarized in Table 4.7.1.1.3-5.

Lower CK levels were observed in all treated males and lower total Bilirubin levels in all treated females compared to controls, but in both cases the absence of dose-relationship and the high control values showed that these were not treatment-related effects. Creatinine and calcium levels were higher (up to +9% and +57%, respectively) in males treated at 500 to 1,000 mg/kg/day, compared to controls. Cholesterol levels were dose-relatedly lower in males treated at 100-1,000 mg/kg/day and high-dose females, and triglycerides levels were dose-relatedly lower in males treated at 10-1,000 mg/kg/day and females treated at 500-1,000 mg/kg/day, all compared to controls. High-dose females showed slightly lower total protein and albumin levels than controls. These last four parameters were most probably influenced in a large extent by the effects of the test item on food intake.

At the end of the recovery period, none of these parameters showed statistical differences between the investigated groups.

Table 4.7.1.1.3-5: Blood biochemistry in metosulam treated and control principal rats.

Dose-level (mg/kg/day)	0		10		100		500		1,000	
Sex	M	F	M	F	M	F	M	F	M	F
Ca^{2+} (mg/dl)	9.57	10.37	10.03	10.61	10.17 *	10.31	10.37 *	10.12	10.43 *	9.93
Creatinine (mg/dl)	0.49	0.64	0.44	0.57	0.47	0.58	0.71*	0.66	0.77*	0.66
Cholesterol (mg/dl)	67.6	102.4	64.3	111.0	50.1*	96.6	47.9*	82.4	41.4*	75.1*
Triglycerides (mg/dl)	94.6	79.6	57.0*	78.4	58.3*	57.6	48.3*	45.6*	44.1*	45.1*
Tot. Proteins (g/dl)	6.3	7.3	6.0	7.1	5.9*	7.2	6.0	6.9	6.1	6.6*
Albumin (g/dl)	3.3	4.7	3.0*	4.7	3.1	4.5	3.2	4.3	3.2	4.0*

*: statistical difference, $p<0.05$

At *post-mortem* examination, testes, adrenal gland and kidney weights showed some minimal variations from control values. These were of statistical but not biological significance: absence of dose-relationship, significance limited to absolute or relative weights, and absence of histological lesions (for testes and adrenals). Kidney weights are yet mentioned in Table 4.7.1.1.3-6, because at the end of the recovery period their relative weights had become significantly lower in treated males than in controls.

Table 4.7.1.1.3-6: Relative kidney weights in metosulam treated and control rats.

Dose-level (mg/kg/day)		0		10		100		500		1,000	
Sex		M	F	M	F	M	F	M	F	M	F
Treatment	Final body weight (g)	550.4	278.3	493.1*	289.9	474.7*	283.9	438.4*	239.7*	414.7*	238.3*
	Kidneys (%)	0.569	0.674	0.608	0.692	0.552	0.609	0.577	0.654	0.533	0.659
Recovery	Final body weight (g)	541.3	322.0	-	-	-	-	-	-	483.9*	282.9*
	Kidneys (%)	0.605	0.646	-	-	-	-	-	-	0.538*	0.640

*: statistical difference, $p<0.05$.

High-dose males showed decreases in the amount of abdominal fat, a finding which can be attributed to the treatment-related reduction of food intake.

Microscopic examination of the proximal tubules in the kidneys showed several treatment-related renal lesions: very slightly to slightly increased basophilia (females treated at 500-1,000 mg/kg/day), slight to moderate epithelial hypertrophy (males treated at 500-1,000 mg/kg/day), very slight to moderate nuclear pleomorphism (both sexes at 1,000 mg/kg/day, less marked in females than in males).

At microscopic examination, satellite animals, kept for 4-week recovery after the 13-week treatment period, only showed nuclear pleomorphism, and its frequency and severity was inferior to those observed in animals killed at the end of the treatment period.

Table 4.7.1.1.3-7: *Post-mortem* macroscopic and microscopic findings: incidence (/10 animals per sex, group and period) in metosulam treated and control rats.

Dose-level (mg/kg/day)			0		10		100		500		1,000	
Sex			M	F	M	F	M	F	M	F	M	F
Treatment	Decreased mesentery fat		0	0	0	0	0	0	0	0	**6**	0
	Kidneys: proximal tubules	Increased basophilia	0	0	0	0	0	0	0	**10**	0	**10**
		Epithelial hypertrophy	0	0	0	0	1	0	**10**	0	**10**	0
		Nuclear pleomorphism	0	0	0	0	**9**	**9**	**10**	**10**	**10**	**9**
Recovery		Nuclear pleomorphism	0	0	-	-	-	-	-	-	**10**	**6**

Conclusion

Dietary administration of metosulam at nominal dose-levels of 10, 100, 500 or 1,000 mg/kg/day to Sprague-Dawley rats for 13 weeks induced:

- minor clinical signs at 1,000 mg/kg/day: hindquarter hair loss (only females), fecal and urine staining (mainly females) of the perineum;
- dose-related lower food intake and body weight, in treated males (100-1,000 mg/kg/day) and females (500-1,000 mg/kg/day) compared to controls, with partial recovery potential; these findings already being significant at the end of week 1 suggests an important unpalatability of administered diets, whereas findings listed below demonstrate renal effects which can account for maintained dysorexia;
- in males at 500-1000 mg/kg/day, minimally lower urine specific gravity than in controls (this finding was present as a trend in females), with complete recovery potential;
- at blood biochemistry: alterations attributable to dysorexia-related lower metabolism (lower cholesterol, triglycerides, total proteins and albumin) and to impaired renal function (higher creatinine and calcium), all parameters showing complete recovery potential;
- minimally lower kidney weights, a finding which was mainly present at the end of the recovery period (animals treated at 1000 mg/kg/day);
- decreased amount of mesentery fat in males (1000 mg/kg/day);
- 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), with null (nuclear pleomorphism in females), partial (nuclear pleomorphism in males) or full (other lesions) recovery potential.

The diets containing the test item were unpalatable to Sprague-Dawley rats. Kidneys were the only target organ. Recovery of renal nuclear pleomorphism was limited (females) or incomplete (males) after a 4-week treatment-free period.

4.7.1.1.4 Barna-Lloyd T, Szabo JR, Rachunek BL (1992): XRD-511 herbicide: Chronic dietary toxicity study in dogs

Four groups of 4 male and 4 female Beagle dogs (age not mentioned, bw range 8.8-10.9 kg for males, 7.2-9.2 kg for females) were administered technical metosulam (batch 275252, purity of 99.1%) in their diet, at dose-levels of 0, 3, 10 and 37.5 mg/kg/day (based on group mean body weight and food intake) for 52 weeks, before final necropsy. The first treatment day was noted day 1.

The animals were observed daily for clinical signs and mortalities. Additional evaluations were performed weekly. Ophthalmology was performed on pre-test and at 3 to 8-week intervals until last examination on day

360. Individual body weights were recorded on pre-test and then every 7 days. Weekly food consumption was determined on pre-test and then every 7 days.

Haematology (red, white blood cells and blood smears) and blood biochemistry were performed on blood samples taken from all animals on pre-test, on days 100, 184 and 359. Urinalysis was performed on all animals at necropsy.

After sacrifice, all animals were subjected to gross *post-mortem* external and internal examination. Selected organs (brain, pituitary, thyroid with parathyroids, adrenals, heart, liver, kidneys, testes/ovaries) were weighed. Histolopathologic examination was performed on selected organs (gross lesions, adrenal glands, aorta, bone, bone marrow, brain, cecum, cervical lymph node, cervix, colon, duodenum, epididymides, esophagus, eyes and optic nerve, gall bladder, heart, ileum, jejunum, kidneys, liver, lungs, mammary glands, mediastinal lymph node, mediastinal tissue, mesenteric lymph node, mesenteric tissue, ovaries, oviducts, pancreas, parathyroid glands, peripheral nerve, pituitary gland, prostate, rectum, salivary gland, skeletal muscle, skin and subcutis, cervical thoracic and lumbar spinal cord, spleen, stomach, testes, thymus, thyroid gland, tongue, tonsil, trachea, urinary bladder, uterus and vagina) from all animals.

Results

Test material concentrations in treatment diets were satisfactory as they were all within ±20% of nominal values. The test item was not detectable in diets prepared for controls.

There were no treatment-related clinical signs. Retinal detachment was diagnosed at ophthalmologic examinations in 2/4 high-dose males (both from day 63) and 2/4 high-dose females (from day 102 and 165), and later confirmed at histopathology (Table 4.7.1.1.4-2).

There were no differences in body weights or food consumption between treated and control animals.

At haematological examinations at the end of the treatment period, platelet counts in high-dose animals were lower than respective controls (males: -38%, females: -12%), including once corrected for pre-test differences (males: -35%, females: -25%) (Table 4.7.1.1.4-1).

Urinalysis performed at the end of the study showed no treatment-related finding.

Blood biochemistry in high-dose animals showed (all variations % are from control values once corrected for pre-test diffrences) lower CK (males: -42%, females: -55%), higher PAL (males: +100%, females: +31%) and creatinine (males: +17%, females: +48%), and high-dose females also showed lower potassium (males: -8%, females: -17%). These differences were considered to be biologically significant and treatment-related.

Table 4.7.1.1.4-1: Incidence (/4 animals per sex and group) of findings at hematology, urinalysis and blood biochemistry in metosulam treated and control dogs.

Dose-level (mg/kg/day)		0		3		10		37.5	
Sex		M	F	M	F	M	F	M	F
Pre-test investigations									
Haematology	Platelet count ($10^3/cm^3$)	350	331	448	418	341	387	335	392
Blood biochemistry	Creatine kinase (U/l)	163	160	182	180	173	230	164	151
	Alcaline phosphatase (U/l)	93	86	131	93	97	95	97	96
	Creatinine (mg/dl)	0.73	0.72	0.84	0.76	0.79	0.60	0.80	0.70
	Potassium (meq/l)	4.89	4.75	4.76	5.26	4.65	5.31	4.84	4.94
Terminal investigations									
Haematology	Platelet count ($10^3/cm^3$)	294	290	303	360	259	356	**183**	**256**
Blood biochemistry	Creatine kinase (U/l)	115	114	104	100	90	76	**67**	**48**
	Alcaline phosphatase (U/l)	47	60	71	73	62	56	**98**	**88**
	Creatinine (mg/dl)	1.14	0.93	1.13	0.97	1.20	0.90	**1.46**	**1.34**
	Potassium (meq/l)	4.67	4.82	4.36	4.54	4.84	4.63	**4.24**	**4.21**

Kidney relative weights were dose-related but showed opposite trends in males (lower when treated, up to -21%) and females (higher when treated, up to +14%). The finding was not considered to be treatment-related in females because the high mean was attributable to one female, showing marked renal vasculature congestion.

There was no treatment-related finding at gross necropsy.

Histopathological findings are summarized in Table 4.7.1.1.4-2. Bilateral retinal degeneration with or without detachment was observed in several high-dose animals (3/4 males, 2/4 females), and was more severe in females. Gallbladder from 3/4 high-dose males and females showed diffuse mucin accumulation within exaggerated mucosal folds. Kidneys from all high-dose animals showed treatment-related lesions. They were multifocal interstitial aggregates of mononuclear cells with non suppurative pyelitis and diffuse urocystitis in 3/4 females, and slight to moderate degeneration localized to distal convoluted ducts or collecting ducts (often with duct fibrosis) in both sexes.

Table 4.7.1.1.4-2: *Post-mortem* microscopic findings: incidence (/4 animals per sex and group) in treated and control dogs.

Dose-level (mg/kg/day)		0		3		10		37.5	
Sex		M	F	M	F	M	F	M	F
Eyes	Bilateral retinal degeneration	0	0	0	0	0	0	**3**	**2**
	Bilateral retinal detachment	0	0	0	0	0	0	**1**	**2**
Gallbladder	Diffuse mucin accumulation within exaggerated mucosal folds	0	0	0	0	0	0	**3**	**3**
Kidneys/urinary	Distal convoluted duct degeneration	0	0	0	0	0	0	**2**	**1**

tract	Bilateral collecting duct degeneration	0	0	0	0	0	0	**4**	**3**
	Slight multifocal collecting duct fibrosis	0	0	0	0	0	0	**3**	**2**
	Multifocal interstitial aggregates of mononuclear cells with non suppurative pyelitis and diffuse urocystitis	0	0	0	0	0	0	0	**3**

Conclusion

Dietary administration of metosulam at dose-levels of 3, 10 or 37.5 mg/kg/day to Beagle dogs for 52 weeks induced the following effects in animals treated at 37.5 mg/kg/day:

- retinal degeneration with or without detachment, diagnosed clinically and at histopathology in 5/8 animals;
- lower platelet counts (roughly, -30%);
- lower CK, higher PAL and creatinine in both sexes, and lower potassium in females;
- mucin accumulation within exaggerated mucosal folds in the gallbladder;
- in the kidneys (and urinary tract): multifocal interstitial aggregates of mononuclear cells with non suppurative pyelitis and diffuse urocystitis in 3/4 females, slight to moderate degeneration localized to distal convoluted ducts or collecting ducts (often with duct fibrosis) in both sexes.

4.7.1.2 *Repeated dose toxicity: inhalation*

No data available.

4.7.1.3 *Repeated dose toxicity: dermal*

No relevant effect at a dose up to 1000 mg/kg/d in the 3-week dermal study in the Rabbit.

4.7.1.4 *Repeated dose toxicity: other routes*

4.7.1.4.1 Szabo JR, Davis NL (1991): XRD-511 herbicide: 14-day ocular irritation and absorption study in beagle dogs

Technical metosulam (batch AGR 275252, purity of 98.9%) was instilled at the dose-level of 0.1g behind the third eyelid of the right eye of 3 male and female Beagle dogs (18 months old, body weight range 13.1-14.5 kg for males, 9.1-10.8 kg for females) every day for 14 days. The test item was removed after one hour contact. The other eye served as an untreated control.

Dogs were submitted to direct ophthalmologic examination before the first treatment. They were observed daily for clinical signs including signs of pain or discomfort. Dogs were weighed before instillation and weekly thereafter. Food intake was determined every week. Every day, both eyes of all dogs were scored for ocular irritation at 1h post-treatment and were submitted to a complete direct ophthalmologic examination at 24h.

After 2 weeks post-treatment, all animals were sacrificed and submitted to a complete gross examination. Histopathology examination was performed on both eyes from all dogs.

Results

Repeated conjunctival instillation of metosulam produced minimal, focal necrosis of the medial supraorbital conjunctiva in 1 female dog (out of 6 dogs) with amelioration over the remaining test period. This transient

necrosis was judged to be the result of mechanical damage to the conjunctiva secondary to incomplete removal of the test material. No treatment-related clinical sign or ocular lesion was observed in any animal.

Conclusion

Repeated conjunctival instillation of metosulam to beagle dogs for 14 days produced no treatment-related effects.

4.7.1.5 *Human information*

No reported incidents of adverse effects during the manufacture or formulation of metosulam.

4.7.1.6 *Other relevant information*

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4.7.1.6.1 Szabo JR, Rachunek BL (1989) : Range-finding and palatability probe study in beagle dogs

Three studies were conducted on 4-month old Beagle dogs treated with technical metosulam (batch AGR 265439, purity of 98.8%). Post-mortem examinations are detailed after study definitions.

1) Range-finding study (gelatin capsules):
One male was administered 1000 mg/kg for one day and then 2,000 mg/kg/day for the next 2 days. Three other males were administered 2,000 mg/kg/day for 5 days. All animals were weighed daily during treatment, and observed daily for clinical signs and mortality for 14 days after the last dosing. They were then subjected to pre-terminal ophthalmology and sacrificed.

2) First palatability study (dietary administration):
Four groups (named 1a to 4a) of 2 females received 0, 250, 500 and 1,000 mg/kg/day for 2 weeks. Animals were observed for clinical signs and mortality daily, and body weights and food consumption were recorded weekly. At the end of the treatment period, one female of each group was sacrificed, while the remaining females were given untreated diet for a 2-week recovery period before pre-terminal ophthalmology and sacrifice.

3) Second palatability study (dietary administration):
Four groups (named 1b to 4b) of 2 male and 2 females received 0, 25, 100 and 250 mg/kg/day for 2 weeks. Animals were observed for clinical signs and mortality daily, and body weights and food consumption were recorded weekly. At the end of the treatment period they were subjected to pre-terminal ophthalmology and sacrificed.

Post-mortem examinations in all animals:
Sacrifice was followed by gross necropsy and by histopathologic examination of the eyes, optic nerve, kidneys, liver, and additionally lungs in case of macroscopic finding.

Results

1) Range-finding study (gelatin capsules):
No clinical sign, ophthalmologic finding, treatment-related body weight change, mortality or post-mortem finding was observed in any animal.

2) First palatability study (dietary administration):

All animals treated at 500-1000 mg/kg/day were slower and weaker than other animals. One mid-dose female was recumbent, showed black stool, and was found dead on day 8. One high-dose female was found dead on day 12.
All females given 250-1000 mg/kg/day showed retinal detachment at pre-terminal ophthalmologic examination.
Dose-related body weight loss was observed during the 2 treatment weeks in all treated animals (see Table 4.7.1.6.1-1 below), due to drastically, dose-relatedly reduced food intake when compared to pre-test. Therefore, actual cumulated dosage was not proportional to target dose-levels, but it was still dose-related.
Recovery of body weight was thereafter partial in females kept for 2 additional weeks.

Table 4.7.1.6.1-1: Mean body weights (g) in metosulam treated and control dogs.

Dose-level (mg/kg/day)	0	250	500	1000
Sex	F	F	F	F
Mean cumulated dosage (g/kg)	0	836	1107	2126
Mean weekly food intake change (%)a	-19	-94	-98	-100
Mean body weight gain (%)a	+1	-17	-22b	-26b

a: change (%) from pre-test to end of week 2
b: represents one animal only because of death of the second one

Post-mortem macroscopic findings included presence of dark ingesta in the digestive lumen (various locations, 500-1000 mg/kg/day), haemorrhagic content in the digestive lumen (various locations), eyes, stomach or lymph nodes (1000 mg/kg/day), and firm lung consistency (250-1000 mg/kg/day).
Post-mortem microscopic findings were (all at 250-1000 mg/kg/day) diffuse bilateral retinal detachment with very severe necrosis and renal degenerative lesions (mononuclear aggregates, degeneration, fibrosis, tubular mineralization and necrosis) of slight to severe intensity.

3) Second palatability study (dietary administration):
One female treated at 250 mg/kg/day was found dead on day 14.

All males and females given 100-250 mg/kg/day showed retinal detachment at pre-terminal ophthalmologic examination.
Dose-related body weight loss was observed during the 2 treatment weeks in all animals treated at 100-250 mg/kg/day (see Table 4.7.1.6.1-2), due to drastically, dose-relatedly reduced food intake when compared to pre-test. Therefore, actual cumulated dosage was not proportional to target dose-levels, but it was still dose-related.

Table 4.7.1.6.1-2: Mean body weights (g) in dogs given metosulam for 14 days.

Dose-level (mg/kg/day)	0		25		100		250	
Sex	M	F	M	F	M	F	M	F
Mean cumulated dosage (g/kg)	0	0	304	300	559	485	581	845
Mean weekly food intake change (%)a	+28	+21	+4	+3	-85	-92	-100	-96b
Mean body weight gain (%)a	+10	+9	+6	+8	-14	-18	-22	-23b

a: change (%) from pre-test to end of week 2
b: represents one animal only because of death of the second one

Post-mortem macroscopic findings were eroded/red colon mucosa (250 mg/kg/day), discoloration of kidneys, liver or lungs (250 mg/kg/day), bilaterally atrophied testes (250 mg/kg/day) and thymus (100-250 mg/kg/day). These effects were yet not considered to be treatment-related as they were not observed in the previous study, at higher dietary dose-levels.
Post-mortem microscopic findings were (all at 100-250 mg/kg/day) diffuse bilateral retinal detachment with very severe necrosis and renal degenerative lesions (degeneration, tubular mineralization and necrosis) of very slight to moderate intensity.

Conclusion

Oral administration of metosulam at dose-levels up to 2,000 mg/kg/day (max. 5 days) in gelatin capsules induced no toxic effect in male Beagle dogs, probably due to lack of absorption of the dose (see 4.1: only 19.2% of an orally administered aqueous solution of metosulam was absorbed by male Beagle dogs).

Dietary administration of metosulam at dose-levels of 25, 100, 250, 500 and 1000 mg/kg/day for 2 weeks (2 studies) induced:

- deaths (250-1000 mg/kg/day),
- clinical signs of weakness (500-1000 mg/kg/day),
- dose-related and drastic reduction of food intake causing body weight loss (100-1000 mg/kg/day) and also lower exposition to the test item than expected,
- presence of dark ingesta in the digestive lumen (various locations, 500-1000 mg/kg/day), hemorrhage of digestive lumen (various locations), eyes, stomach or lymph nodes (1000 mg/kg/day all), and firm lung consistency (250-1000 mg/kg/day),
- diffuse bilateral retinal detachment (100-1000 mg/kg/day) with very severe necrosis,
- renal degenerative lesions (mononuclear aggregates, degeneration, fibrosis, tubular mineralization and necrosis) of very slight to severe intensity (100-1000 mg/kg/day).

4.7.1.6.2 Yano BL, Breslin WJ, Liberacki AB (1990): XRD-511: Oral gavage range finding study in New Zealand white rabbits

Groups of 3 non-pregnant adult female New Zealand white rabbits (age not mentionned, bw ranging 2.9-3.3 kg) were orally treated with technical metosulam (batch AGR 275252, purity of 98.9%) at the dose-levels of 0 (controls), 300, 600 or 1000 mg/kg/day (based on measured body weights), by gavage under a dose-volume of 2 ml distilled water /kg. The treatment was performed during 13 days.

The animals were observed daily for clinical signs and mortality. Each animal was weighed daily.
Surviving animals were sacrificed at the end of the treatment period and their liver and kidneys were weighed. After terminal sacrifice or premature sacrifice/death, all animals were subjected to gross necropsy and microscopic examination of liver, kidneys and gallbladder.

Results

Achieved concentrations were satisfactory (within $\pm$20% of target concentrations, except on rare occasions for the dose-level of 300 mg/kg/day; no metosulam was detected in control formulations). Homogeneity was satisfactory.

Rabbits given 600 or 1 000 mg/kg/day either died or were moribund prior to the completion of the study.

No clinical sign was observed in rabbits treated at 300 mg/kg/day. At higher dose-levels, anorexia and soiling appeared from day 7 or 9 until death or sacrifice.

Almost all rabbits given 600 or 1000 mg/kg/day lost weight during the first 7 days.

Absolute and relative kidney weight was markedly higher in the only surviving mid-dose animal, when compared to controls (+41%, +91%, respectively), although terminal weight was markedly lower in this animal (-26%).

Table 4.7.1.6.2-1: Summary of treatment-related findings in female rabbits treated orally with metosulam for 13 days (3 rabbits/dose-level).

Dose-level (mg/kg/day)	0	300	600	1000
Mortality	0	0	**2**	**3**
Mean body weight gain (g, days 1-7)[a]	84.5	43.1	**-16.8**	**-72.2**
Terminal body weight (g)	3194	3217	**2356**[b]	a
Absolute kidney weight (g)	15.5	16.2	**21.9**[b]	a
Relative kidney weight (%)	0.49	0.51	**0.93**[b]	a

a: no/not enough survivor(s) for terminal investigations (weight, weight gain and organ weights)

b: based on one survivor

Treatment-related kidney and gallbladder lesions occurred in rabbits given metosulam. Kidney lesions were identified in one rabbit given 300 mg/kg/day and all of the rabbits given 600 or 1 000 mg/kg/day and consisted primarily of tubular epithelial cell degeneration and necrosis. Focal necrosis and inflammation of the gallbladder occurred in the majority of rabbits given 600 or 1 000 mg/kg/day.

Conclusion

Oral administration of metosulam to female rabbits at dose-levels up to 1 000 mg/kg/day induced (compared to controls):

- Higher mortality, preceded by soiling and anorexia, and subsequently lower body weight gains, at dose-levels of 600 and 1000 mg/kg/day;
- Markedly higher (+91% relative) kidney weights at 600 mg/kg/day (no high-dose animal was weighed), with renal tubular epithelial cell degeneration and necrosis at 300 (very slight, 1/3 animals) to 1000 mg/kg/day (slight to moderate, systematic);
- Focal necrosis and inflammation of the gallbladder at 600-1000 mg/kg/day.

No NOAEL could therefore be set under the experimental conditions of the study. The dose-level of 300 mg/kg/day was considered as a LOAEL based on slight renal lesions observed at histological examination.

4.7.1.6.3 Makin A, Lanham DF, Gregson RL, Offer JM, Gopinath C (1990): XRD-511 - 6-week preliminary oral toxicity study in cynomolgus monkeys

Two male and one female cynomolgus monkeys (1.5-3 year old, 2.0-3.1 kg) were given metosulam (batch 275252, purity of 98.9%) as a suspension in corn oil, by daily oral gavage so as to reach the dose-level of 100 mg/kg/day, for 6 weeks. A similar group made of one male and two females received the vehicle alone and acted as a control group.

All animals were blood sampled on pre-test and on weeks 3 (prior to dosing) and 6 (1h post-dosing) for pharmacokinetics. Daily, at regular intervals throughout the working day, all animals were checked for

clinical signs and mortality. All animals were weighed on pre-test and then at the end of each week. Daily food intake was recorded for each animal from beginning of week 2 until study termination. All animals were submitted to complete direct ophthalmologic examinations on pre-test and on weeks 3 and 6.

At terminal sacrifice, all animals were examined for gross abnormalities and their eyes and kidneys were histologically examined. Samples from the vitreous from the eyes of all animals were analyzed for presence of the test item.

Results

The test item was not detectable (below LOQ of 2.87 µg/ml) in the serum of control animals. The test item levels detected in the serum of treated animals were markedly higher on week 6 compared to week 3 (successive mean levels: 37.5 and 90.8 µg/ml, i.e. +142%), and were sensibly different between the 3 treated animals (more than 3-fold differences on week 3). The increase was most probably attributable to differences in sampling time-points (prior to dosing on week 3, 1h post-dosing on week 6) and therefore not considered to be relevant.

Over the treatment period, treated animals showed a higher total incidence of pale feces (+68% days present) and diarrhoea (+194% days present) than controls. This was considered to be biologically significant and treatment-related.

Over the treatment period, treated animals showed minimally lower mean body weights gains (-58g, controls: +94g) and mean food consumption (-9% from controls) than controls. Yet this was attributable to only one treated male (noted Mb in the table below), which had an abscess and consequently lower appetite and body weight loss. These minimal differences were therefore not considered to be treatment-related.

Table 4.7.1.6.3-1: Treatment-related differences between control and treated cynomolgus monkeys over a 6-week period

Dose-level (mg/kg/day)	0				100				Treated/ controls (%)
Sex and animal	M1	F1	F2	Group mean	Ma	Mb	Fa	Group mean	
Incidence[z] of pale feces	11	22	27	20	35	33	33	34	+68
Incidence[z] of diarrhoea	7	3	8	6	28	18	7	18	+194
Body weights gains (g)	+104	+93	+86	+94	+4	-294	+115	-58	ND
Mean[y] weekly food consumption (g)	770	780	926	825	746	670	826	747	-9

z: total number of days present, out of 42 days (1 animal*7days*6 weeks) ND: not determined

y: mean over 5 weeks as data for week 1 were lost.

There were no treatment-related findings at ophthalmoscopy and at macroscopic and microscopic post-mortem examination. The test item was not detectable (below LOQ of 0.260 µg/ml) in the vitreous of any animal.

Conclusion

The repeated oral administration of metosulam to the cynomolgus monkey at 100 mg/kg/day for 6 weeks produced an increased frequency of pale feces and diarrhoea. There were no other treatment-related findings reaching biological significance. Exposure to the test item was demonstrated by the plasmatic levels obtained (mean on week 6, 1h post-dosing: 90.8 µg/ml).

4.7.1.6.4 Yano BL, Haut KT (1992): XDE-51: 7-day dietary study to evaluation the temporal development of nephrotoxicity in male Sprague-Dawley rats

The purpose of this study was to evaluate the very early changes in the development of kidney lesions in male rats given metosulam in food. These data will assist in establishing appropriate time points for which the kidneys of rats could be examined in a more detailed 2-week dietary study.

Four groups of 2 male Sprague-Dawley rats (6 weeks old, body weight range 176-222g) were given diets formulated with 100 mg/kg bw/day of metosulam (batch AGR 275252, purity of 99.4%) for 1, 3, 5, or 7 days, based on mean body weights and food intake. Rats were clinically examined and body weights and food consumption data were obtained. After 1, 3, 5, or 7 days of exposure to metosulam, the kidneys of 2 rats/time interval were examined for gross and histopathologic alterations.

Results

There were no abnormal in-life observations noted in any of the rats. Body weights and food consumption increased during the study at expected rates. Based on actual body weight and food consumption data, these rats received 85.6 to 106.0 mg metosulam/kg/day with a group mean of 98.6 mg/kg/day, very close to the targeted concentration of 100 mg/kg/day.

Histopathologic examination revealed no lesions in the kidneys of rats necropsied following 1 or 3 days of treatment with metosulam. Necrosis of individual renal tubular epithelial cells occurred in 1/2 rats given metosulam for 5 days and 2/2 rats given metosulam for 7 days (with respectively focal and multifocal extension). In addition, the rats given metosulam for 7 days showed multifocal degeneration and regeneration of tubular epithelial cells. Degeneration/regeneration involved approximately 5 to 8% of the renal cortical parenchyma in the region of the proximal convoluted tubules and was graded as slight. It was characterized by the presence of slightly vacuolated, more basophilic epithelial cells and usually involved all epithelial cells in the cross-section of an affected tubule. Occasional mitotic figures and nuclear enlargement (karyomegaly) were also present. Necrosis of individual cells involved less than 5% of cortical tubular epithelial cells and was graded as very slight. Necrotic cells usually occurred in regions of epithelial cell degeneration/regeneration.

Table 4.7.1.6.4-1: Microscopic findings in kidneys of rats exposed for 1, 3, 5 or 7 days to metosulam (2 animals/group).

Duration of exposure (days)		1	3	5	7
Renal tubular epithelial cell lesions	Very slight necrosis	0/2	0/2	1/2 (focal)	2/2 (multifocal)
	Slight degeneration/regeneration	0/2	0/2	0/2	2/2 (multifocal)

Conclusion

Treatment-related effects were not identified in the kidneys of male rats given 100 mg/kg/day for 1 or 3 days. Renal tubular necrosis and degeneration/regeneration of tubules occurred in rats given 100 mg /kg/day for 5 or 7 days. These lesions were very slight to slight and did not affect any of the other parameters investigated.

4.7.1.6.5 Yano BL, Haut KT (1992): XDE-511: 2-week dietary toxicity study in male and female Sprague-Dawley rats to evaluate early renal tubular epithelial cell lesions

Four groups of 10 male and 10 female Sprague-Dawley rats (6 weeks old, body weight ranges 164-193g for males, 131-157g for females) were given diets formulated with 0 (controls), 5, 30 or 100 mg/kg/day of metosulam (batch AGR 275252, purity of 99.4%), based on mean body weights and food intake. One half of each group (5 animals/sex/dose-level) was sacrificed after 7 days of treatment, and the other half after 14 days of treatment.

The animals were observed at least twice daily for clinical signs and mortality. Individual body weight and food consumption were recorded in surviving animals on pre-test and on days 7 and 14.
All animals were blood sampled prior to necropsy for renal blood chemistry investigations (urea nitrogen and creatinine concentrations), and urinalysis (including microscopic examination of urine sediment) was performed. At necropsy kidneys were weighed and examined for gross abnormalities and microscopic lesions.

Results

There were no treatment-related clinical signs, mortality or differences in body weights or food consumption between treated and control animals. Actual ingested dose-levels, calculated from body weights and food intake, were well correlated with target dose-levels (within ± 15% of target values).

There were no differences in urine specific gravity. Increased numbers of white blood cells occurred in the urine sediment of male and female rats given 100 mg/kg/day for 1 and 2 weeks. Increases in the number of epithelial cells also occurred in the sediment of male and female rats given 100 mg/kg/day for 2 weeks. There were no statistically identified or biologically significant differences for blood urea nitrogen and creatinine values for male and female rats given metosulam in the feed for up to 2 weeks.

There were no statistically identified differences in kidney weights, and no treatment-related gross abnormality.

Treatment-related histopathologic effects which occurred in the kidneys of male and female rats given 100 mg/kg/day for 1 and 2 weeks are summarized in Table 4.7.1.6.5-1. Alterations attributed to metosulam in male and female rats given 100 mg/kg/day for 1-week included the following tubular epithelial cell alterations: degeneration/regeneration and necrosis of individual cells, increased mitotic figures, increased nuclear pleomorphism. These findings were all localized to the proximal part of the convoluted tubule in males, and to the descending part of the proximal tubule in females. Based on lesion frequency and severity, females were slightly more affected than males.

Table 4.7.1.6.5-1**:** Histopathologic observations at 1-week sacrifice in male and female rats (/5 animals/sex/dose-level)

Group		1		2		3		4	
Dose-level (mg/kg/day)		0		5		30		100	
Sex		M	F	M	F	M	F	M	F
Proximal tubule, descending part									
Degeneration/regeneration	very slight	**0**	1	0	2	1	0	0	0
	moderate	**0**	0	0	0	0	0	0	**5**
Increased mitotic figures	very slight	**0**	0	0	0	0	0	0	**2**
Increased nuclear pleomorphism	very slight	**0**	0	0	0	0	0	**0**	**4**
Necrosis, individual cells	very slight	**0**	0	0	0	0	0	0	**3**
Convoluted tubule, proximal part									
Degeneration/regeneration	very slight	**3**	4	3	4	3	4	0	0
	slight	**0**	0	0	0	0	0	**4**	0
	moderate	**0**	0	0	0	0	0	**1**	0
Increased mitotic figures	very slight	**0**	0	0	0	0	0	**3**	0
Increased nuclear pleomorphism	very slight	**0**	**0**	0	0	0	0	**4**	0
Necrosis, individual cells	very slight	**0**	0	0	0	0	0	**4**	0
	slight	**0**	0	0	0	0	0	**1**	0

Histopathologic lesions identified in the kidneys of male and female rats given metosulam for 2 weeks were qualitatively similar in appearance, distribution and severity to those noted following 1 week of exposure to metosulam (Table 4.7.1.6.5-2). The differences from 1-week examinations were:

- the incidence and severity of tubular degeneration/regeneration was lower at 2 weeks
- female rats given 30 mg/kg/day for 2 weeks had a very slight occurrence of individual epithelial cell necrosis that was not seen at 1 week
- The incidence of mitotic figures was no more increased in comparison to controls at 2 weeks.

Table 4.7.1.6.5-2**:** Histopathologic observations at 2-week sacrifice in male and female rats (/5 animals/sex/dose-level)

Group		1		2		3		4	
Dose-level (mg/kg/day)		0		5		30		100	
Sex		M	F	M	F	M	F	M	F
Proximal tubule, descending part									
Degeneration/regeneration	very slight	**1**	3	0	2	0	3	0	0
	slight	**0**	0	0	0	0	0	0	**5**
Increased nuclear pleomorphism	very slight	**0**	0	0	0	0	0	**0**	**5**
Necrosis, individual cells	very slight	**0**	0	0	0	0	**2**	0	**5**
Convoluted tubule, proximal part									
Degeneration/regeneration	very slight	**5**	2	3	5	3	5	4	5
	slight	**0**	1	0	0	0	0	1	0
Increased nuclear pleomorphism	very slight	**0**	**0**	0	0	0	0	**5**	0
Necrosis, individual cells	very slight	**0**	0	0	0	0	0	**4**	0

Treatment-related kidney lesions were not observed in male rats given 5 or 30 mg/kg/day for 2 weeks or female rats given 5 mg/kg/day for 2 weeks.

Conclusion

Treatment-related effects occurred in the kidneys of male and female rats given 100 mg/kg/day, and to a much lesser extent in females given 30 mg/kg/day. These effects were very slight to moderate in severity and consisted in tubular epithelial cell degeneration/regeneration, increased mitotic figures, individual cell necrosis and nuclear pleomorphism. They were accompanied by minor alterations in the urine sediment, but did not affect the other investigated parameters (health status, body weight, feed consumption, kidney weights, serum urea nitrogen and creatinine).

4.7.1.6.6 Yano BL, Haut KT, Redmond JM (1992): XDE-511: 2-week dietary toxicity study in male and female Sprague-Dawley rats to quantify renal tubular epithelia cell mitosis using bromodeoxyuridine

The data from the previous studies indicate that the kidney is the primary organ in rats affected by XDE-511. The purpose of this study was to examine whether there was any indication of increased epithelial cell turnover preceding the development of the nuclear pleomorphism and kidney tumours.

Two groups made of 10 male and 10 female Sprague-Dawley rats (6 weeks old, body weight ranges 158-183g for males, 136-148g for females) were given diets formulated with 0 (controls) or 100 mg/kg/day of metosulam (batch AGR 275252, purity of 99.4%), based on mean body weights and food intake. Osmotic pumps containing a 20 mg/ml solution of bromodeoxyuridine (BrdU), implanted in the inter-scapular subcutaneous tissue, delivered a constant infusion at the rate of 10 µl/h for 7 days until scheduled necropsy. One half of each group (5 animals/sex/dose-level) was sacrificed after 7 days of treatment, and the other half after 14 days of treatment.

The animals were observed at least twice daily for clinical signs and mortality. Individual body weight and food consumption were recorded in surviving animals on pre-test and on days 7 and 14.
At necropsy kidneys were weighed and examined for gross abnormalities and microscopic lesions. A piece of duodenum from each rat was also microscopically examined. Histopathological examinations were performed after standard coloration (one slide/rat) and also after a specific immunohistochemical technique localizing incorporated BrdU (one slide/rat). BrdU incorporation into renal tubular epithelial cells was subjectively graded using a light microscope based on the approximate percent of nuclei which appeared dark brown to black according to the following criteria: very slight - ≤ 25%, slight - > 25% to ≤ 50%, moderate - > 50% to ≤ 75% and severe - > 75%. BrdU incorporation into the duodenal epithelium was only graded as being present or absent (positive control for BrdU incorporation).

Results

There were no treatment-related clinical signs, mortality or differences in body weights or food consumption between treated and control animals. Actual ingested dose-levels, calculated from body weights and food intake, were well correlated with target dose-levels (within ± 12% of target values).

There were no treatment related differences in kidney weights or in gross lesion type and frequency between treated and control animals.

Treatment-related effects which occurred in the kidneys of male and female rats given 100 mg/kg/day for 1 and 2 weeks are summarized in Tables 4.7.1.6.6-1 and 4.7.1.6.6-2. Lesions identified in the kidneys of male and female rats given 100 mg/kg/day for 2 weeks were similar to those seen following 1 week of exposure. However, in females a number of differences were noted following 2 weeks versus 1 week of exposure and included:

- The incidence of mitotic figures was no more increased in comparison to controls at 2 weeks.
- A lower incidence/severity of tubular degeneration/ regeneration in the medulla at 2 weeks
- An apparent treatment-related extension of tubular degeneration/regeneration into the cortex by involving the proximal convoluted tubules at 2 weeks.

Table 4.7.1.6.6-1**:** Histopathologic observations at 1-week sacrifice in male and female rats (/5 animals/sex/group)

Dose-level (mg/kg/day)		0		100	
Sex		M	F	M	F
Proximal tubule, descending part					
Degeneration/regeneration	very slight	1	0	3	0
	moderate	0	0	0	**5**
Increased nuclear pleomorphism	very slight	0	0	0	**5**
Necrosis, individual cells	very slight	0	0	0	**5**
Convoluted tubule, proximal part					
Degeneration/regeneration	very slight	2	2	0	0
	slight	0	0	**5**	0
Increased nuclear pleomorphism	very slight	0	0	**5**	0
Necrosis, individual cells	very slight	0	0	**4**	0
	slight	0	0	**1**	0

Table 4.7.1.6.6-2: Histopathologic observations at 2-week sacrifice in male and female rats (/5 animals/sex/group)

Dose-level (mg/kg/day)		0		100	
Sex		M	F	M	F
Proximal tubule, descending part					
Degeneration/regeneration	very slight	0	0	0	2
	moderate	0	0	0	**3**
Increased nuclear pleomorphism	very slight	0	0	0	**5**
Necrosis, individual cells	very slight	0	0	0	**5**
Convoluted tubule, proximal part					
Degeneration/regeneration	very slight	3	3	0	1
	slight	0	0	**5**	3
Increased nuclear pleomorphism	very slight	0	0	**5**	0
Necrosis, individual cells	very slight	0	0	**5**	0

BrdU incorporation was present in all duodenum slides. It was evenly distributed within and between the renal cortex and medulla of male and female rats. In male and female rats given 100 mg/kg/day for 1 week, a slight increase in the degree of BrdU incorporation occurred in tubular epithelial cells of the cortex (Table 4.7.1.6.6-3). BrdU incorporation in both male and female rats given 100 mg/kg/day involved tubular epithelial cells in regions of degeneration/regeneration.

Table 4.7.1.6.6-3: BrdU incorporation at 1-week sacrifice in male and female rats (/5 animals/sex/group)

Group		1		2	
Dose-level (mg/kg/day)		0		100	
Sex		M	F	M	F
Cortex					
Intranuclear incorporation	Very slight	5	5	0	5
	Slight	0	0	5	0
Medulla					
Intranuclear incorporation	Very slight	5	5	5	0
	Severe	0	0	0	5

The extent of BrdU incorporation in the kidneys of male rats given 100 mg/kg/day for 2 weeks was similar to that observed following 1 week of exposure. However, in females given 100 mg/kg/day for 2 weeks the amount of BrdU incorporation was lower than that observed at 1 week (Table 4.7.1.6.6-4).

Table 4.7.1.6.6-4**:** BrdU incorporation at 2-week sacrifice in male and female rats (/5 animals/sex/group)

Group		1		2	
Dose-level (mg/kg/day)		0		100	
Sex		M	F	M	F
Cortex					
Intranuclear incorporation	Slight	0	0	5	0
Medulla					
Intranuclear incorporation	Very slight	5	5	5	0
	Moderate	0	0	0	4
	Severe	0	0	0	1

Conclusion

Treatment of male and female Sprague-Dawley rats with 100 mg metosulam/kg/day by dietary admixture for 1 or 2 weeks caused significant renal toxicity, which consisted of tubular epithelial cell necrosis, degeneration/regeneration, increased mitoses, and increased nuclear pleomorphism. BrdU incorporation into epithelial cell nuclei in regions of tubular epithelial cell toxicity was increased above control levels in male and female rats given 100 mg/kg/day for 1 and 2 weeks. In conclusion, the results of this study indicate that a significant degree of renal tubular epithelial cell degeneration, necrosis, and regeneration occur in the first 1 and 2 weeks of exposure to 100 mg metosulam kg/day and that these lesions develop prior to the occurrence of nuclear pleomorphism and kidney tumours.

4.7.1.7 *Summary and discussion of repeated dose toxicity (Refer to Vol. 1 of Additional report on metosulam, 2009)*

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.

Oral toxicity studies:

- *CD1 mice :*

Metosulam was administered daily to CD1 mice by dietary admixture at the dose-levels of 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 was 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-related and time-related severity in animals given the test item at doses $\geq$ 1000 mg/kg/day. Slightly decreased liver vacuolation was also observed in both sexes given 5000 mg/kg/day for 2 weeks, and focal necrosis and inflammation of the liver in 2/10 males given the test item at 2000 mg/kg/day for 13 weeks. Slightly higher incidence of renal tubule degeneration/regeneration was observed in male mice given the test item at 2000 mg/kg/day for 13 weeks.

- *Rats* :

Metosulam was administered daily to Sprague Dawley rats by dietary admixture at the dose-levels of 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 the dose-levels of 0, 1000 or 5000 mg/kg/day for 2 weeks.

No target organs were identified after 2-week 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 $\geq$ 100 mg/kg/day and females treated at $\geq$ 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 $\geq$ 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 $\geq$ 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 the dose-levels of 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 $\geq$ 250 mg/kg/day, associated with clinical signs of weakness, and dose-related severe reduction of food intake causing body weight loss at doses $\geq$ 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, renal collecting duct degeneration were observed at dose-levels $\geq$ 100 mg/kg/day after 2 weeks of treatment, $\geq$ 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 liver from females treated at 50/mg/kg/day for 13 weeks, and mucin accumulation within exaggerated mucosal folds in the gallbladder in animals treated at 37.5 mg/kg/day for 52 weeks, both associated with higher PAL 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. No NOAEL could therefore be set, however the dose-level of 300 mg/kg/day was considered as a LOAEL based on slight renal lesions observed at histological examination.

Repeated dermal application study:

- *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 (which is a solid). There were therefore no significant treatment-related findings. The NOAEL was 1,000 mg/kg/day in both sexes.

Sensitivity to the toxic potential of metosulam was highest in dogs and lowest in CD1 mice. Metosulam toxicity is mainly detected at *post-mortem* examinations, with limited functional impairment, except for reduced body weight gain and retinal detachment causing blindness in dogs. Target organs are:

- the kidneys (mainly renal tubule degeneration/regeneration) in mice, rats, dogs and rabbits;
- the eyes, with severe lesions of retinal detachment and necrosis, observed in dogs only and leading to blindness;

- and to a much lesser extent the liver in mice and dogs, and the gallbladder in dogs and rabbits.

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 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, in the range of those observed in dogs (150 µg equivalent/mL including almost exclusively unchanged metosulam, at 4 hours after a single administration).

4.7.1.8 *Summary and discussion of repeated dose toxicity findings relevant for classification according to DSD*

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 lesions was also observed at higher doses in repeated dose toxicity studies in Rat (13-week, 2-generations & 24 month studies), Rabbit (2-week and developmental studies) and Mouse (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. They may be related to the accumulation of metosulam in the tapetum and in the retina of the dog (Timchalk, C.; Dryzga M. D.; Johnson, K. A.; Eddy, S.L.; Freshour, N. L.; Nolan, R.J. (1992)).

However, the mechanism of action is unknown. Consequently, extrapolation from animal to humans remains unclear.

4.7.1.9 *Comparison with criteria of repeated dose toxicity findings relevant for classification according to DSD*

Rationale for classification as R48/22 (Danger of serious damage to health by prolonged exposure):

The 67/548/EEC criteria for classification as R48/22 are as follow:
Substances are classified as R48/22 when significant serious damage (clear functional disturbance or morphological change which has toxicological significance), is likely to be caused by repeated or prolonged exposure by an appropriate route. As a reference, substances are classified at least as harmful when these effects are observed at levels of the order of $\leq$ 50 mg/kg/day in an oral 90-day repeated-dose study conducted in rat. . When interpreting the results of a sub-acute (28-days) toxicity test, this value should be increased approximately three fold. If a chronic (two-years) toxicity test is available, it should be evaluated on a case-by-case basis. If results of studies of more than one duration are available, then those from the study of the longest duration should normally be used.

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/d in the sub-acute studies, Metosulam has to be classified Xn, R48/22 according to Directive 67/548/EEC.

4.7.1.10 *Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification according to DSD*

A classification R48/22 (according to Directive 67/548/EEC) is proposed.

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

4.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

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 lesions was also observed at higher doses in repeated dose toxicity studies in Rat (13-week, 2-generations & 24 month studies), Rabbit (2-week and developmental studies) and Mouse (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. They may be related to the accumulation of metosulam in the tapetum and in the retina of the dog (Timchalk, C.; Dryzga M. D.; Johnson, K. A.; Eddy, S.L.; Freshour, N. L.; Nolan, R.J. (1992)).

4.8.2 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

However, the mechanism of action is unknown. Consequently, extrapolation from animal to humans remains unclear.Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

Rationale for classification as STOT-RE :

The CLP criteria for classification as STOT-RE are as follow:
“Substances are classified in category 2 for target organ toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were observed in a 90-day repeated-dose study conducted in experimental animals within the guidance value ranges of 10-100 mg/kg/d.
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 greater or lesser 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.
For studies with exposure durations shorter than 9 days (i.e 10% of the 90 days to which the default general guidance value applies) the guidance value used should be no greater than 10 times the default guidance

value. For example, the effects in an oral range-finding study of 9 days or less should be compared with a guidance value of 1000 mg/kg bw/day for STOT-RE Category 2.

Based on the Haber's rule, the relevant renal and ocular lesions listed in point 4.8.1, are observed within the reference 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, what would lead to a category 1 classification. However, for this specie/strain, metosulam would not be classified according to the same conversion rule, based on the two-generation reproduction (18-weeks administration) and the two-year chronic studies, in which rats were tested at the same doses.

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

Table 4.8.1-1 : Summary of the converted minimal doses inducing renal and ocular lesions according to Haber's rule.

Study (1)	**Reference**	**Conversion as if it was a 13-week study**	**Category for target organ toxicity**
Oral, 2 weeks, Beagle dogs 25, **100**, 250, 500, 1,000 mg/kg/d (dietary administration, 2 weeks) 2000 mg/kg/d (gelatin capsules, 5 days)	Szabo JR, Rachunek BL (1989)	**Renal and ocular lesions** : ≥ 15 mg/kg bw/d	**Category 2**
Oral (gavage), 2 weeks, NZW female rabbits **300**, 600, 1000 mg/kg/d	Yano BL, Breslin WJ, Liberacki AB (1990)	**Renal lesions** : ≥ 46 mg/kg bw/d	**Category 2**
Oral (diet), 13 weeks, Beagle dogs 5, 25, 50 mg/kg/d	Szabo JR, Davis NL (1990)	**Renal lesions** : ≥ 25 mg/kg bw/d **Ocular lesions** : At 50 mg/kg bw/d	**Category 2**
Oral (diet), 13 weeks, CD-1 mice 250, 1000, 2000 mg/kg/d	Szabo JR, Rachunek BL (1989)	**Renal lesions** : At 2000 mg/kg bw/d only	**Not classified**
Oral (diet), 13 weeks, SD rats 10, 100, 500, 1000 mg/kg/d	Szabo JR, Grandjean M (1989)	**Renal lesions** : ≥ 100 mg/kg bw/d:	**Category 2**
Oral (diet), 52 weeks, Beagle dogs, 3, 10, **37.5** mg/kg/d	Barna-Lloyd T, Szabo JR, Rachunek BL (1992)	**Renal and ocular lesions** : At 150 mg/kg/d	**Not classified**
Oral, 2-week, Sprague-Dawley rats 5, **30**, 100 mg/kg/d	Yano BL, Haut KT (1992)	**Renal lesions** : ≥ 5 mg/kg/d	**Category 1**
Oral, 2-week, Sprague-Dawley rats 100 mg/kg/d	Yano BL, Haut KT, Redmond JM (1992)	**Renal lesions** : 15 mg/kg/d	**Category 2**
Oral, Two-year chronic dietary toxicity/oncogenicity study, Sprague-Dawley rats, 5, **30** or 100 mg/kg/day	Zempel JA, Grandjean M, Campbell RA, Szabo JR (1992)	**Renal lesions** : ≥ 240 mg/kg/d	**Not classified**

Oral, dietary oncogenicity study, CD-1 mice 30, 300 or 1000 mg/kg/day	Barna-Lloyd T, Szabo JR, Campbell RA, Davis NL (1992	**Renal lesions** : 8000 mg/kg/d	**Not classified**
Two-generation dietary reproduction study in Sprague-Dawley rats 5, 30 and **100** mg/kg bw/d	Zempel JA, Mensik DC, Szabo JR (1991)	**Renal lesions** : 140 mg/kg/d (2)	**Not classified**
Oral gavage teratology study in New Zealand white rabbits 30, **100**, 300 mg/kg bw/d	Liberacki AB, Breslin WJ, Yano BL (1990)	**Renal lesions** : ≥ 15 mg/kg/d (3)	**Category 2**

(1) ***Figures in bold :*** *minimal doses inducing renal and ocular lesions*
(2) Considering that the duration of a two-generation reproduction study in rodents is about 18 weeks.
(3) Considering that the duration of a teratology study is about 15 days.

4.8.3 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

A classification STOT-RE. 2 – H373 (kidney, eye) is proposed. The only route of exposure available for the species affected by the toxic effects, on which this classification is based, is oral route.

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):
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.

New Zealand white rabbits (Dermal):
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

Rationale for classification as STOT-RE 2:
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 ≤ 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.

4.9 Germ cell mutagenicity (Mutagenicity)

Table 16. Summary table of relevant in vitro and in vivo mutagenicity studies

Method	Results	Remarks	Reference
Gene mutation Ames test 1 *S typhimurium* (TA 1535, TA 1537, TA 98 and TA 100) *OECD 471 (1981)*	**Negative**	Purity : 99.1 %. Strains TA102 of *S. typhymurium* or uvrA, wpr2 of *E. coli* were not tested.	Samson YE, Gollapudi BB (1990)
Gene mutation Ames test 2 *S typhimurium* (TA 1535, TA 1537, TA 98, TA 100 and TA 102) *OECD 471*	**Negative**	Purity : 98.4%	Herbold B. (2007)
***In vitro* mammalian cell gene mutation test** Chinese hamster ovary cell *OECD N° 476 (1981)*	**Negative**	Purity : 98.6 - 98.8 %	Linscombe VA, Gollapudi BB (1990)
***In vitro* mammalian cell gene mutation test** Chinese hamster V79 cells *OECD N° 476 (1997)*	**Negative**	Purity : 98.8 %. No repetition with metabolic activation. However, the results are concordant with the CHO test and the study is therefore considered as acceptable.	Wollny HE (2002)
***In vitro* chromosomal aberration test** Chinese hamster V79 cells *OECD N° 473 (1997)*	**Negative**	Purity : 98.8 %	Schulz M (2002)
***In vitro* chromosomal aberration test** Rat lymphocytes *OECD 473 (1983)*	**Negative**	Purity : 99.1 %	Linscombe VA, Mensik DC, Sinha AK (1992)
***In vitro* UDS Test** Rat hepatocytes *US EPA (1983). Procedures described by Williams G.M. Cancer Res. 37:1845-1851 (1977), comparable to OECD Guideline 482.*	**Negative**	Purity : 99 %	McClintock ML,Gollapudi BB (1989)
***In vivo* micronucleus test** Male and female CD1 mice *OECD guideline 474 (1981)*	**Negative**	Purity : 99.1 %. 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 20%.	Gollapudi BB, Samson YE, McClintock ML (1990)
***In vivo* comet assay** Male rat renal cells	**Negative**	Purity : 98.4 %. An OECD guideline is currently under consideration for the comet assay. However,	Wirnitzer U. (2007)

Method	Results	Remarks	Reference
		the study followed the recommendations for an appropriate performance of the assay using OECD guidelines for other *in vivo* tests and followed the standard protocol and acceptance criteria for the assay developed through the International Workshop on Genotoxicity Working Parties and international Comet assay workshops (Tice et al., 2000).	

4.9.1 Non-human information (Refer to Vol. 1 of Additional report on metosulam, 2009)

4.9.1.1 *In vitro data*

Technical metosulam had no mutagenic effect on the TA1535, TA1537, TA98 and TA100 strains of Salmonella typhymurium in a plate incorporation assay conducted according to the ames test procedure at dose-levels up to 100.0 µg/plate, without and with metabolic activation (Samson YE., Gollapudi BB.; 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 B.; 2007) and 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 labeling of primary rat hepatocytes when tested up to the limit of solubility in comparison to solvent controls.

4.9.1.2 *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 on 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 U.; 2007) 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.

4.9.2 Human information

No reported incidents of adverse effects during the manufacture or formulation of metosulam.

4.9.3 Other relevant information

No data available.

4.9.4 Summary and discussions of mutagenicity

Metosulam induced no genotoxic effect in *in vitro* and *in vivo* tests.

4.9.5 Comparison with criteria

The CLP criteria for classification of substances for substances which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans (Category 2), are as follow :
– Positive evidence obtained from experiments in mammals and/or in some cases from *in vitro* experiments, obtained from:

- Somatic cell mutagenicity tests *in vivo*, in mammals; or
- Other *in vivo* somatic cell genotoxicity tests which are supported by positive results from *in vitro* mutagenicity assays.

Considering that metosulam induced no genotoxic effect in *in vitro* and *in vivo* tests, no classification is required for metosulam under either Directive 67/548/EEC or the CLP Regulation.

4.9.6 Conclusions on classification and labelling

No classification is required for metosulam under either Directive 67/548/EEC or the CLP Regulation.

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) 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.

4.10 Carcinogenicity

Table 17. Summary table of relevant carcinogenicity studies

Method	Results	Remarks	Reference
Oral, Two-year chronic dietary toxicity/oncogenicity study, Sprague-Dawley rats, 5, 30 or 100 mg/kg/day	- Higher WBC counts in high-dose males, with a slight shift from lymphocytes to neutrophils; - lower urine specific gravity in high-dose males and females treated at 30 and 100 mg/kg/day; - at gross necropsy, higher frequency of kidney nodules or masses in high-dose males (18/50); - at histopathology, higher frequency of non 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 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)		Zempel JA, Grandjean M, Campbell RA, Szabo JR (1992)

Oral, dietary oncogenicity study, CD-1 mice 30, 300 or 1000 mg/kg/day	- minimally lower kidney weights (males -17%, females -10%) in mice administered 1 000 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)		Barna-Lloyd T, Szabo JR, Campbell RA, Davis NL (1992

4.10.1 Non-human information

4.10.1.1 *Carcinogenicity: oral*

4.10.1.1.1 Zempel JA, Grandjean M, Campbell RA, Szabo JR (1992): XRD-511 (herbicide): Two-year chronic dietary toxicity/oncogenicity study in Sprague-Dawley rats

A total of 240 male and 240 female Sprague-Dawley rats (8 weeks old, bw ranging 216-309 g for males and 164-231 g for females) were randomly allocated to 3 groups of 60 males and 60 females given technical metosulam (batch GW 1468850, lot WP890227, purity of 98.9 to 99.4% along the study) in the diet in order to obtain dose-levels of 5, 30 or 100 mg/kg/day for 2 years, based on group mean body weights and food intake. Another group of 60 males and 60 females (with same characteristics) received the untreated diet and served as a control group. The first treatment day was noted day 1. After 12 months, interim sacrifice was performed on 10 animals per sex and dose-level including controls, the remaining animals being designated as "terminal animals".

The animals were observed at least once daily for clinical signs and mortality. Detailed observations were conducted weekly. All animals were palpated for externally detectable masses on pre-test, prior to the interim sacrifice and monthly thereafter. Each animal was weighed at weekly intervals for the first 3 months and then every month. Mean food consumption was calculated from 20 animals/group, every week for the first 13 weeks and then once a month, and food conversion ratio was calculated from these animals every week for the first 13 weeks.
Surviving animals were blood sampled at 6 and 12 months (all interim animals) or at 18 and 24 months (10 terminal animals/sex/group) for standard haematological (including blood smear examination) and blood chemistry investigations. Urinalysis was performed at the same time-points on the same animals.
Animals found dead, killed prematurely or at the end of the 104 weeks of treatment were weighed and subjected to gross necropsy. Organ weights (adrenals, brain, liver, kidneys, heart, ovaries/testes) were only determined after scheduled death. Selected tissues were preserved and histological examinations were performed on specified tissue samples (gross lesions, adrenals, aorta, auditory sebaceous glands, bone with marrow, brain, cecum, cervix, coagulating glands, colon, duodenum, epididymides, esophagus, eyes with optic nerves, heart, ileum, jejunum, kidneys, lacrimal/harderian glands, larynx, liver, lungs, mammary glands, mediastinal and mesenteric lymph nodes and tissues, nasal turbinates, oral tissues, ovaries, oviducts, pancreas, parathyroid glands, peripheral nerve, pituitary, prostate, rectum, salivary gland, seminal vesicle, skeletal muscle, skin and subcutis, cervical/thoracic/lumbar spinal cord, spleen, sternum, stomach, testes, thymus, thyroid gland, tongue, trachea, urinary bladder, uterus, vagina) from all controls and high-dose animals. Adrenals, kidneys, liver, lungs and gross lesions were also examined in all other animals.

Results

The clinical observations (daily and detailed weekly observations) did not indicate any treatment-related findings.

Analysis of cumulative mortality did not show any significant difference between treated and control animals. Major death causes in male rats administered 0, 5, or 30 mg/kg/day were pituitary gland neoplasms, myocardial degeneration and chronic progressive glomerulonephropathy, and pituitary gland neoplasms and renal adenocarcinomas in males given 100 mg/kg/day. In all groups of females, the most common causes of

mortality were pituitary gland neoplasms, and to a lesser extent large, often necrotic, mammary gland neoplasms. These death causes are common in this strain of rats.

Table 4.10.1.1.1-1: Cumulative mortality and percentage of mortality in controls and metosulam treated terminal groups (50 animals/sex/group)

Dose-level (mg/kg/day)	0		5		30		100	
Sex	Males	Females	Males	Females	Males	Females	Males	Females
months 1-13	3	1	2	1	1	3	0	4
months 1-24	28	29	21	28	27	31	27	32

No statistical difference.

Incidence and type of cutaneous or subcutaneous neoplasms noted in males and females were judged to be typical/within the normal range of the Sprague-Dawley rat (reference cited: Lang 1987), and no treatment-related trend was established.

Food intake, mean body weights and food conversion ratios were not treatment-related in males and females along the study.

Hemoglobin (HGB) and/or packed cell volume (PCV) values were statistically significantly lower than in controls, in high-dose males sampled at 6 (-5% HGB, -4% PCV) and/or 12 months (-7% PCV) and in high-dose females sampled at 6 months (-7% HGB). Some other statistical differences were noted, without any dose-relationship. As these haematological changes were of low amplitude and only significant during the first year of the study, i.e. in animals which had been randomly assigned to interim sacrifice, they were considered not to be biologically significant.

In males, white blood cell counts showed statistically significant differences between high-dose and control groups. The high WBC counts during the second year were considered to be treatment related in consideration to its high amplitude, and to the presence of a slight shift from lymphocytes to neutrophils, compared to controls. This may suggest an exacerbation of geriatric inflammatory lesions in high-dose males.

Table 4.10.1.1.1-2: White blood cells: absolute and differential counts in controls and metosulam treated interim and terminal groups (max. 10 animals/sex/group/time-point).

Dose-level (mg/kg/day)		0		5		30		100	
Sex		M	F	M	F	M	F	M	F
End of 1st year (interim animals)									
White blood cell count	Absolute (10^3/mm^3)	11.3	8.1	10.6	7.2	9.8	9.1	8.2*	8.5
	Neutrophils (%)	28	26	33	21	25	31	29	29
	Lymphocytes (%)	68	70	62	75	73	66	68	68
End of 2nd year (terminal animals)									
White blood cell count	Absolute (10^3/mm^3)	9.6	7.6	10.4	6.8	8.9	7.5	**17.6***	9.2
	Neutrophils (%)	41	46	57	49	48	36	**52**	51
	Lymphocytes (%)	57	53	41	49	49	63	**46**	48

*: statistical difference, $p<0.05$.

Total bilirubin and creatine phosphokinase (CK) levels were the main biochemical changes observed throughout the study in both males and females given 30 or 100 mg/kg/day (Table 4.10.1.1.1-3). Although the lower total bilirubin values (up to -52%) appeared to be treatment-related, they were not associated with any histopathologic changes in the liver. Moreover, this parameter showed a large degree of variability within dose groups, and stayed within normal limits of historical ranges obtained in this strain of rats.

In males, CK values for treated groups were generally lower than control (up to -48%) at most time intervals. However, these changes were minimal and of no biologic or toxicologic significance due to the lack of correlative skeletal or cardiac muscle alterations.

Other occasional biochemical changes were observed (albumin, globulin, glucose, alanine amino transferase, aspartate amino transferase, cholesterol, glycerides) in males or females. Nevertheless, none of these changes were dose-related nor consistent among the evaluation periods, they were thus considered as incidental and of no toxicological significance.

Table 4.10.1.1.1-3**:** Mean values of selected blood biochemistry parameters at 24-month in male and female rats given metosulam by dietary admixture

Dose-level (mg/kg/day)	0		5		30		100	
Sex	M	F	M	F	M	F	M	F
Total bilirubin	**0.24**	0.27	0.19	0.23	**0.15***	**0.21***	**0.17***	**0.13***
Creatine phosphokinase	**611**	420	**268***	**217***	555	263	433	368

*: statistical difference, $p<0.05$.

At the 18-month evaluation the mean urine specific gravity of both males and females given 100 mg/kg/day was significantly lower compared to controls (Table 4.10.1.1.1-4). At 24-month evaluation, the urine specific gravity of the males given 100 mg/kg/day was slightly lower but not statistically different compared to controls. Also, both the 30 and 100 mg/kg/day females had significantly lower urine specific gravity. Although the magnitude of these changes was small, the slightly lower urine specific gravity may represent a decreased ability to concentrate urine secondary to the histopathologic changes observed in the kidneys of these animals.

Table 4.10.1.1.1-4: Mean urine specific gravity values at 18- and 24-month in male and female rats

Dose-level (mg/kg/day)	0		5		30		100	
Sex	M	F	M	F	M	F	M	F
18-month	**1.070**	1.056	1.058	1.057	1.057	**1.042**	**1.047 ***	**1.038 ***
24-month	**1.047**	1.060	1.052	1.045	1.044	**1.039 ***	**1.030**	**1.034***

*: statistical difference, $p<0.05$.

There were no statistically significant differences in relative organ weights at the 12- or 24-month necropsies. The higher mean relative kidney weights observed in high-dose males (+235% when compared to controls) was due primarily to two animals that had extremely high kidney weights due to the presence of large renal neoplasms. The same finding was present to a much lesser extent in high-dose females, and explained by the presence of a small mass diagnosed as an adenocarcinoma in one kidney.

Table 4.10.1.1.1-5**:** Mean kidney weights at 24-month in male and female rats

Dose-level (mg/kg/day)	0		5		30		100	
Sex	M	F	M	F	M	F	M	F
Absolute weight (g)	**5.441**	3.102	4.956	3.139	4.414	3.023	**10.659**	3.543
Relative weight (%)	**0.882**	0.691	0.784	0.756	0.730	0.652	**2.073**	0.818

No statistical difference.

At *post-mortem* gross examination no treatment related findings were observed in interim animals. At study termination, a higher frequency of kidney nodules or masses was observed in high-dose terminal males (18/50 compared to 0/50).

Non neoplastic lesions:

At the 12-month sacrifice, the histopathological lesions attributable to metosulam administration consisted of a dose-related increase in the frequency and severity of proximal tubule epithelial cell nuclear pleomorphism in male and female rats. In males, nuclear pleomorphism was graded as very slight at 30 mg/kg/day to moderate at 100 mg/kg/day. In females, nuclear pleomorphism was graded as very slight at 30 mg/kg/day to slight at 100 mg/kg/day. The altered morphology of affected nuclei was characterized by anisokaryosis, peripheral condensation of nuclear chromatin, prominent nucleoli, and abnormal mitotic figures.

The same finding was also present in animals sacrificed after 24 months of treatment, at the dose-levels of 30 and 100 mg/kg/day in both sexes. High-dose terminal males also showed renal epithelial hyperplasia localized to tubules (multifocal) or to a much lesser extent to the pelvis (diffuse). This may have been a response to renal toxicity of the test item and could in some cases be a pre-neoplastic lesion.

All other non-neoplastic lesions were found at very low and non dose-related frequencies and were similar to those commonly found in this strain of rats.

Table 4.10.1.1.1-6**:** Group distribution of non-neoplastic microscopic findings in kidneys from rats treated with metosulam for 1 or 2 years

Dose-level (mg/kg/day)		0		5		30		100	
Sex		M	F	M	F	M	F	M	F
End of 1st year (/10 interim animals/column)									
Proximal tubule epithelial cell nuclear pleomorphism		0	0	0	0	**3**	**4**	**10**	**10**
End of 2nd year (/50 terminal animals/column)									
Proximal tubule epithelial cell nuclear pleomorphism		0	0	0	0	**30***	**26***	**47***	**44***
Epithelial hyperplasia	tubules, multifocal	1	0	0	0	2	0	**22***	2
	pelvis, diffuse	0	2	0	0	0	1	**6***	2

Neoplastic lesions:

The incidence of neoplastic lesions is summarized in Table 4.10.1.1.1-7.

In high-dose males, the proportion of animals with primary malignant tumors and the total number of these tumors was higher compared to controls (no statistical comparison performed). These findings were not observed in females. The incidence of benign tumors was not dose-related.

Only kidneys from high-dose males, and to a lesser extent, females, showed significantly higher tumor incidence compared to controls. Two tumors were observed at a moderate to high frequency: basophilic cortical adenomas (only as a trend in males, non significant in females), basophilic cortical adenocarcinomas (with or without metastasis, significant in males, trend in females). On the whole, the total number of rats with primary renal neoplasms was significantly higher in both sexes in the high-dose group compared to controls.

In females, mammary tumor incidence was negatively correlated with dose-levels (not in table). Other tumors were observed at incidences within the reported range of normal and were not considered to be treatment-related.

Table 4.10.1.1.1-7: Group distribution of primary neoplastic lesions and renal tumors in rats treated with metosulam for 2 years (/50 terminal animals/column)

Dose-level (mg/kg/day)		0		5		30		100	
Sex		M	F	M	F	M	F	M	F
General incidence (excluding renal adenomas and adenocarcinomas)									
Rats with primary neoplasms (/50)	total	39	49	41	49	39	48	48	47
	malignant	12	23	9	20	15	15	**32**	17
Total number of primary malignant neoplasms		12	23	10	20	15	16	**36**	19
Detailed observations in kidney (number of affected rats/50)									
Basophilic renal cortex adenomas		0	0	0	0	0	0	**4^{t}**	2
Basophilic renal cortex adenocarcinomas	without metastasis	0	0	0	0	0	0	**8***	**4^{t}**
	with metastasis	0	0	0	0	0	0	**9***	0
Total number of animals with at least one primary renal neoplasm		0	0	1	0	1	0	**20***	**6***

*: statistical difference, $p<0.05$; t: only statistical linear trend, $p<0.05$

Table 4.10.1.1.1-8: Comparison between values reported by Lang (1984 and 1992, raw data not available) and Cohen et al. (1979, raw data not available) for tumour incidences, and control and high dose tumour incidences for males in this study

Alteration	Incidences for male in this study (1989-1992)		Incidences from the references cited in the study report		
	Control male	High dose male	Lang (1987)	Lang (1992)	Cohen (1979)
Pituitary gland adenoma	40.0	46.0	26.3-50.0	37.1-81.3	20.0
Pituitary gland adenocarcinoma	18.0	18.0	0-24.7	1.0-33.3	No data
Mammary gland adenoma	0.0	0.0	0-1.5	1.1-2.8	No data
Mammary gland fibroadenoma	2.0	8.0	0-16.7	1.6-25.0	No data
Mammary gland adenocarcinoma	0.0	0.0	0-5.6	1.6-4.7	No data
Integument	2.0	4.0	0-3.7	1.4-8.6	No data

papilloma					
Integument squamous cell carcinoma*	0.0	8.0	0-3.3	1.1-4.0	No data
Zymbals gland carcinoma	No data		No data	1.1-3.9	No data
Keratoacanthoma	4.0	4.0	0-6.8	1.4-14.0	No data
Trichoepithelioma	0.0	2.0	0-6.8	1.4-2.9	No data

** includes zymbals gland squamous cell carcinoma*

Conclusion

The long term toxic and carcinogenic effects of metosulam to rats were evaluated following dietary administration at the concentrations of 0, 5, 30 and 100 mg/kg/day for up to 104 weeks. Treatment-related effects in terminal animals (killed at the end of the 2nd year) were (compared to controls):

- higher WBC counts in high-dose males, with a slight shift from lymphocytes to neutrophils;
- lower urine specific gravity in high-dose males and females treated at 30 and 100 mg/kg/day;
- at gross necropsy, higher frequency of kidney nodules or masses in high-dose males (18/50);
- at histopathology, higher frequency of non 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 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).

Based on the results of this study, **metosulam has a carcinogenic effect on kidneys from rats** treated at 100 mg/kg/day, especially in males. The kidneys are also the target organ for long-term oral toxicity in rats treated at 30 or 100 mg/kg/day. The NOAELs are therefore 30 mg/kg/day for carcinogenicity and 5 mg/kg/day for chronic toxicity, in both sexes.

4.10.1.1.2 Barna-Lloyd T, Szabo JR, Campbell RA, Davis NL (1992): XRD-511 (herbicide) : Dietary oncogenicity study in CD-1 mice

A total of 240 male and 240 female CD1 mice (8 weeks old, bw ranging 24.0-35.6 g for males and 18.0-28.1 g for females) were randomly allocated to 3 groups of 60 males and 60 females given technical metosulam (batch GW 1468850, lot WP890227, purity of 98.9%) in the diet in order to obtain dose-levels of 30, 300 or 1000 mg/kg/day for 18 months, based on group mean body weights and food intake. Another group of 60 males and 60 females (with same characteristics) received the untreated diet and served as a control group. The first treatment day was noted day 1. After 12 months, interim sacrifice was performed on 10 animals per sex and dose-level including controls, the remaining animals being designated as “terminal animals”.

The animals were observed at least once daily for clinical signs and mortality. Detailed observations were conducted twice a week. All animals were palpated for externally detectable masses on pre-test, prior to the interim sacrifice and monthly thereafter. Each animal was weighed at weekly intervals for the first 13 weeks and then every month. Mean food consumption was calculated from 20 animals/group, every week for the first 13 weeks and then once a month, and food conversion ratio was calculated from these animals every week for the first 13 weeks.
Surviving animals were blood sampled at 12 months (all interim animals) or at 18 months (20 terminal animals/sex/group) for standard haematological investigations (including blood smear examination).

After death, all animals were weighed and subjected to gross necropsy. Organ weights (adrenals, brain, liver, kidneys, heart, testes) were only determined after scheduled death. Selected tissues were preserved and histological examinations were performed on specified tissue samples (gross lesions, adrenals, aorta, bone with marrow, brain, cecum, cervix, coagulating glands, colon, duodenum, epididymides, esophagus, eyes with optic nerves, gallbladder, heart, ileum, jejunum, kidneys, lacrimal/harderian glands, larynx, liver, lungs, mammary glands, mediastinal and mesenteric lymph nodes and tissues, nasal tissue, oral tissues, ovaries, oviducts, pancreas, parathyroid glands, peripheral nerve, pituitary, prostate, rectum, salivary gland, seminal vesicle, skeletal muscle, skin and subcutis, cervical/thoracic/lumbar spinal cord, spleen, stomach, testes, thymus, thyroid gland, tongue, trachea, urinary bladder, uterus, vagina) from all controls, high-dose, prematurely killed and decedent animals. Gallbladder, kidneys, liver, lungs and gross lesions were also examined in all other animals.

Results

The clinical observations (daily and detailed weekly observations) did not indicate any treatment-related findings. The most common observations were related to clinical dermatitis, most frequently localized to the ventral neck, ears, and caudal head, attributed to either *Staphylococcus* or *Streptococcus* infections independent from metosulam treatment.

Analysis of cumulative mortality in interim or terminal animals did not show any significant difference between treated and control animals. Most of the deaths prior to scheduled interim necropsy were caused by bacteraemia associated with ventral neck dermatitis. Major death causes before sacrifice of terminal animals were septicaemia, renal disease and systemic amyloidosis (secondary) considered to be attributable to the consequences of clinical and subclinical bacterial dermatitis.

Table 4.10.1.1.2-1: Cumulative mortality and percentage of mortality in controls and metosulam treated terminal groups (50 animals/sex/group).

Group	1		2		3		4	
Dose-level (mg/kg/day)	0		30		300		1000	
Sex	M	F	M	F	M	F	M	F
months 1-12	2	1	3	1	9	1	6	2
months 1-18	26	18	25	11	34	16	29	15

No statistical difference.

Palpable masses were infrequent in any dose group, and not dose-related. The incidence and type of cutaneous and subcutaneous neoplasms were within the normal range for the CD1 mouse (Maita et al., 1998, data not communicated).

In male mice given 300 and 1 000 mg/kg/day, occasional lower body weights were observed. Nevertheless, these differences from control were considered to be incidental due to the lack of a dose response relationship and the high degree of variability seen in all dose groups, including controls. Among the female groups, no statistically, toxicologically, or biologically significant body weight differences from control were found. Administration of metosulam did not affect food consumption or food conversion ratio.

There were no treatment-related findings at haematology.

Absolute kidney weights were lower in male (-17%) and female (-10%) mice administered 1 000 mg/kg/day (Table 4.10.1.1.2-2). At the 18-month sacrifice, the difference in absolute kidney weights in males and females given 1 000 mg/kg bw/day reached statistical significance. These changes were considered to be

treatment-related, but due to the minor differences from control, they were considered to have minimal toxicological significance.

Table 4.10.1.1.2-2: Mean kidney weights at 18-month in male and female mice

Dose-level (mg/kg/day)	0		30		300		1000	
Sex	M	F	M	F	M	F	M	F
Absolute weight (g)	0.852	0.594	0.842	0.569	0.804	0.550	**0.709***	**0.537***
Relative weight (%)	1.918	1.535	1.967	1.466	1.889	1.433	**1.737**	**1.424**

At the 12-month sacrifice, no gross lesions attributable to administration of metosulam were observed in male or female mice at any dose level group. At the 18-month terminal sacrifice, kidneys from high-dose females more often showed an irregular cortical surface than kidneys from controls (respectively: 10/50 compared to 2/50).

Non neoplastic lesions:

At the 12-month interim sacrifice, no histopathologic lesions attributable to dietary administration of metosulam were found in male or female mice of any dose level group.

At the 18-month terminal sacrifice, no histopathologic lesions attributable to dietary administration of metosulam were found in male or female mice at any dose level group.

Tumour Incidence

Administration of metosulam in the daily diet over a period of 18 months had no demonstrable effect on tumour incidence in male or female mice.

Table 4.10.1.1.2-3: Group distribution of primary neoplastic lesions in mice treated with metosulam for 18 months (/50 terminal animals/sex/group)

Group		1		2		3		4	
Dose-level (mg/kg/day)		0		30		300		1000	
Sex		M	F	M	F	M	F	M	F
Mice with primary neoplasms (/50)	total	18	20	24	19	11	20	19	19
	malignant	6	8	5	7	4	6	5	7

No statistical differences.

Table 4.10.1.1.2-4: Group distribution of organ-specific primary tumour incidences in mice treated with metosulam for 18 months.

Dose-level (mg/kg/day)	0		30		300		1000	
Sex	M	F	M	F	M	F	M	F
CERVIX								
Hemangioma	-	**1**/50	-	0/50	-	0/50	-	0/50
Leiomyoma	-	**1**/50	-	**1**/50	-	0/50	-	0/50
Histiocytic sarcoma	-	0/50	-	**1**/50	-	0/50	-	0/50
LACRYMAL GLAND (Cystadenoma)	**4**/50	0/50	0/25	0/10	0/33	**1**/15	**1**/50	**3**/50
LIVER								

Benign hepatocellular tumour	**5**/50	0/50	**12**/50	0/50	**3**/50	**4**/50	**9**/50	**2**/50
Malignant hepatocellular tumour	**1**/50	0/50	**1**/50	**1**/50	**1**/50	0/50	**3**/50	0/50
Hemangioma	**1**/50	0/50	0/50	**1**/50	**1**/50	**2**/50	**1**/50	0/50
LUNGS								
Benign bronchioalveolar tumour	**6**/50	**9**/50	**11**/49	**7**/50	**4**/49	**6**/50	**5**/50	**6**/50
Malignant bronchioalveolar tumour	**3**/50	**1**/50	**1**/49	0/50	0/49	0/50	0/50	**1**/50
MAMMARY GLAND								
Adenoma	-	0/50	-	0/11	-	0/15	-	**1**/50
Adenocarcinoma	-	0/50	-	**1**/11	-	0/15	-	0/50
MEDIASTINAL LYMPH NODE (Lymphosarcoma)	0/50	**1**/50	0/25	0/10	0/32	0/15	0/50	**1**/50
MESENTERIC LYMPH NODE								
Benign tumour	0/41	**1**/47	0/24	0/12	0/33	0/15	0/44	**1**/49
Malignant tumour	0/41	0/47	0/24	**1**/12	**1**/33	**1**/15	0/44	0/49
OVARIES (Hemangioma)	-	0/49	-	**2**/50	-	**2**/50	-	0/48
PANCREAS (Islet cell adenoma)	0/50	0/50	0/25	0/10	0/33	0/15	**1**/50	0/50
SKELETAL MUSCLE								
Hemangiosarcoma	0/50	0/50	**1**/25	0/10	0/33	0/15	0/50	0/50
Osteosarcoma	0/50	0/50	**1**/25	0/10	0/33	0/15	0/50	0/50
SKIN AND SUBCUTIS								
Fibroma	0/50	0/49	0/26	0/11	**1**/36	0/16	0/50	0/50
Hemangioma (popliteal lymp node)	0/50	0/49	0/26	0/11	0/36	0/16	0/50	**1**/50
Carcinoma (clitoral gland)	0/50	0/49	0/26	0/11	0/36	0/16	0/50	**1**/50
Fibrosarcoma	0/50	0/49	0/26	0/11	**1**/36	**1**/16	**1**/50	0/50

M-squamous cell carcinoma	0/50	**1**/49	0/26	0/11	0/36	0/16	0/50	0/50
SPLEEN								
Histiocytic sarcoma	0/50	0/50	0/28	**1**/17	0/33	**1**/19	0/50	0/50
Lymphosarcoma	**2**/50	**2**/50	0/28	**1**/17	0/33	**2**/19	**1**/50	0/50
THYMUS (Lymphosarcoma)	0/50	0/50	**1**/25	**1**/11	**1**/33	**1**/15	0/50	**4**/50 (of which 2 are metastatic)
UTERUS								
Hemangioma	-	**1**/50	-	**1**/50	-	0/50	-	**1**/50
Leiomyoma	-	0/50	-	**1**/50	-	**1**/50	-	0/50
Polyp	-	0/50	-	0/50	-	**1**/50	-	0/50

Conclusion

The long term toxic and carcinogenic effects of metosulam to mice for up to 18 months were evaluated following dietary administration at the concentrations of 0, 30, 300 and 1000 mg/kg/day. Treatment-related effects in terminal animals (killed at the end of the 2nd year) were (compared to controls):

- minimally lower kidney weights (males -17%, females -10%) in mice administered 1 000 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).

Based on the results of this study, metosulam had a mild (no histopathologic lesions) long-term toxic effect on kidneys at the dose-level of 1000 mg/kg/day, and no carcinogenic potential in CD1 mice.

4.10.1.2 *Carcinogenicity: inhalation*

No inhalation data have been reported for metosulam.

4.10.1.3 *Carcinogenicity: dermal*

No dermal data have been reported for metosulam.

4.10.2 Human information

No reported incidents of adverse effects during the manufacture or formulation of metosulam.

4.10.3 Other relevant information

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4.10.4 Summary and discussion of carcinogenicity (Refer to Vol. 1 of Additional report on metosulam, 2009)

The long-term toxicity and carcinogenicity of metosulam was evaluated in dogs, rats and mice exposed orally to metosulam for 1.5 or 2 years.

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

Dietary administration of metosulam to CD1 mice at the concentrations of 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 1 000 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 a mild (no histopathologic lesions) long-term toxic effect on kidneys at the dose-level of 1000 mg/kg/day.

In the same study, metosulam had no carcinogenic potential.

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 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). 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 is nuclear pleomorphism, epithelial cell hypertrophy and cytoplasmic basophilia of epithelial cells (see study in SD rats by Szabo & Grandjean, 1989).

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 can be hypothesized 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, metosulam can be considered as a non-genotoxic carcinogen and should be classified as a **category 3 carcinogen.**

4.10.5 Comparison with criteria

Rationale for classification as a Carcinogen:

The CLP criteria for classification as a category 2 Carcinogen (category 3 carcinogen according to Directive 67/548/EEC) are as follow :
“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. Such evidence may be derived either from limited evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies.”Beyond the determination of the strength of evidence for carcinogenicity, a number of other factors need to be considered that influence the overall likelihood that a substance poses a carcinogenic hazard in humans. Some important factors which may be taken into consideration, when assessing the overall level of concern are:
- tumour type and background incidence;
- multi-site responses;
- progression of lesions to malignancy;
- reduced tumour latency;
- whether responses are in single or both sexes;
- whether responses are in a single species or several species;
- structural similarity to a substance(s) for which there is good evidence of carcinogenicity;
- routes of exposure;
- comparison of absorption, distribution, metabolism and excretion between test animals and humans;
- the possibility of a confounding effect of excessive toxicity at test doses;
- mode of action and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity.

Consequently, considering the following elements:

- 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 Rat paralleled the occurrence of renal tumours and may be the morphologic precursor of renal tumours. This does not seems to occur in cynomolgus monkeys.

- It can be hypothesized 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, consistent with the regenerative epithelial cells noted histologically in males and females,

It can be concluded that based on the observation of treatment-related renal cortical adenomas and adenocarcinomas observed in males and females rats, metosulam should be classified at least as category 2 Carcinogen (category 3 carcinogen according to Directive 67/548/EEC).

Based on the fact that evidence of carcinogenicity is restricted to a single experiment/specie and to a single site, based on the existence of a threshold for the observation of tumours, on the lack of 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 Directive 67/548/EEC).

4.10.6 Conclusions on classification and labelling

A classification as Carc. Cat. 2 – H351 (category 3 carcinogen according to Directive 67/548/EEC) is proposed. The only route of exposure available for chronic studies, is oral route.

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). 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).
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).

4.11 Toxicity for reproduction (Refer to Vol. 1 of Additional report on metosulam, 2009)

Table 18. Summary table of relevant reproductive toxicity studies

Method	Results	Remarks	Reference
Two-generation dietary reproduction study in Sprague-Dawley rats 5, 30 and 100 mg/kg bw/d *OECD 416 (1982)*	Systemic toxicity on adults : - slightly lower food intake and/or body weight gain in males and/or females treated at 100 mg/kg/day during pre-mating, post-mating, gestation and lactation. Reduced appetence was probably in cause. - Histopathologic kidney alterations and decreased kidney weights in the F0 and F1 adults treated at 100 mg/kg/day. Effect on reproduction : No relevant effect.	Purity : 99.2%. Adults : Estrous cycles were not studied. Many usual organ weights (brain, liver, spleen, pituitary, thyroid, adrenals) and moreover weights of testis and epididymides were not recorded. Pups/Young : Age of vaginal opening or preputial separation, weights of brain, spleen and thymus weights were not determined in any animal. No microscopic examination was performed in any animal, i.e. gross lesions and moreover known target organs (kidneys, eyes) and reproductive organs were not examined for histological lesions.	Zempel JA, Mensik DC, Szabo JR (1991)
Dietary teratology study in Sprague-Dawley rats 100, 500 and 1000 mg/kg bw/d *OECD N° 414 (1981)*	Systemic toxicity on adults : Slightly lower body weight gain at 500 and 1000 mg/kg/day. Effect on development : No relevant effect.	Purity : 98.9%.	Liberacki AB, Breslin WJ, Yano BL (1990)
Oral gavage teratology study in New Zealand white rabbits 30, 100, 300 mg/kg bw/d *OECD N° 414 (1981)*	Systemic toxicity on adults : Mortality in 3/24 rabbits given 300 mg/kg/day, necrosis and inflammation of gallbladders, degenerative kidney lesions of moderate severity, and very slight to moderate renal tubule cell necrosis in some rabbits given 100 or 300 mg/kg/day. Effect on development : No relevant effect.	Purity : 98.9%.	Liberacki AB, Breslin WJ, Yano BL (1990)

*: in the preliminary rabbit teratology study only

4.11.1 Effects on fertility

4.11.1.1 Non-human information

Zempel JA, Mensik DC, Szabo JR (1991): XRD-51: Two-generation dietary reproduction study in Sprague-Dawley rats - The Dow Chemical Company, Freeport, TX, USA.

A total of 120 male and 120 female Sprague-Dawley rats (7-8 weeks old, bw 264±10 g for males and 174±10 g for females) were randomly allocated to 3 groups of 30 males and 30 females given technical metosulam (batch AGR 275252, purity of 99.2%) at dietary concentrations calculated to obtain dose-levels of 5, 30 or 100 mg/kg/day (based on mean body weight and food intake), or to a control group given untreated food. Treatment was maintained throughout two successive generations. The first treatment day was noted D0.

F0 adults were treated over a 10-week pre-mating period, throughout the 3-week mating period, gestation and 21-day lactation of 2 litters (F1A and F1B). They were killed after weaning of the pups.

- 30 pups/sex/dose-level were selected from the F1A litter, exposed to metosulam for 12 weeks (they then became F1 adults) and mated to produce the F2 litter. They were killed after weaning of the pups.
- Each litter (F1A, F1B and F2) was randomly culled to 8 pups on day 4 *post-partum* (pp). Weaning was performed on day 21 pp and followed by sacrifice of all pups except for the selected F1A pups (future F2).

All animals were observed daily for mortality and clinical signs. Detailed clinical observations were performed once a week. Decedents (at any age) were submitted to gross external examination.

Adults:

Weekly food intake was determined starting on pre-test for all F0 and F1 adults, except during mating periods. They were weighed at the same time-points, and on days 1, 4, 7, 14 and 21 of lactation.

After sacrifice, all F0 and F1 adults were submitted to complete gross *post-mortem* examination. Kidney weights were measured. Histological examination was performed on gross lesions of all adults, and on kidneys and reproductive organs/tissues (males: epididymides, prostate, testes, seminal vesicles and coagulating glands; females: uterus, ovaries, cervix, oviducts, vagina) taken from control and high-dose animals (and additionally from other groups in case of any potentially treatment-related finding).

Pups and young rats:

Each litter (F1A, F1B and F2) was evaluated for alive and dead pups on delivery, and presence of gross abnormalities. Clinical signs, litter size, number of live and dead pups, sex of the pups and body weights were recorded on days 1, 4, 7, 14 and 21 of lactation. All culled pups and 10 killed weanlings/sex/dose-level were submitted to complete gross *post-mortem* examination. The remaining killed weanlings were only examined for external abnormalities.

Results

The test item was not detected in control diets. Achieved dietary concentrations were satisfactory (within ±20% of target values).

No treatment-related observations or mortalities were recorded for any adult or pup group.

The mean body weights and weight gains of the F0 and F1 males receiving 100 mg/kg/day metosulam in their diet appeared consistently lower (up to -12% bw gain, only significant at some time-points in F1 males during the pre-mating period) than that of their concurrent controls throughout the study (Table 4.11.1.1-3). This was more marked for F1 males at the end of the pre-mating period and during the mating period, at which time-points body weight gains were also affected in low- and mid-dose males. During these periods, body weights were unaffected by the treatment in females.

Table 4.11.1-1: Mean body weight gains (g) in F0 and F1 adults over the pre-mating and mating periods

Sex	males				females			
Dose level (mg/kg/day)	0	5	30	100	0	5	30	100
F0- premating (day 70)	246.5	249.0	243.3	**230.5**	87.2	89.4	96.3	88.3
F1-premating (day 84)	336.6	**306.5***	**305.3***	**292.3***	140.2	131.6	129.5	128.0
F0- post F1A mating (day 140)	326.2	320.1	311.3	301.2	-	-	-	-
F0- post F1B mating (day 217)	351.8	342.8	332.9	329.7	-	-	-	-
F1 – post F2 mating (day 147)	416.9	380.0	388.0	**372.2***	-	-	-	-

* $p < 0.05$ significantly different from controls

During the gestation periods for F1a, F1b and F2 litters, the female 100 mg/kg/day groups always showed lower body weight and weight gain values than concurrent controls, correlating with variations of food intake. These variations were most often significant (7 times/9). Body weights gains of low- and mid-dose females were also affected by the treatment on some occasions for the last 2 gestations (F1b and F2, in bold characters in Table 4.11.1.1-2). However as the F1a gestations were never unaffected at 5 or 30 mg/kg/day), this was not considered to be a biologically relevant finding at these dose-levels.

During the lactation, occasionally statistically significant differences in either body weights or weight gains were identified in high-dose females. Yet these differences were not consistent along the three lactation periods, dose-relationship was inconstant, and body weight gains were not significantly lower so these findings were considered not to be biologically relevant.

Table 4.11.1.1-2: Mean body weight gain (g) in F0 and F1 females over the gestation periods

Dose Level (mg/kg/day)	0			5			30			100		
Week of gestation	1	2	3	1	2	3	1	2	3	1	2	3
FO, F1A gestation	27.4	51.7	131.4	23.3	46.4	122.1	23.9	46.3	123.0	**17.1***	**38.2***	121.9
FO, F1B gestation	26.2	54.4	134.5	19.5*	45.4*	135.6	19.7*	44.5*	128.8	**20.0***	**41.2***	**123.5***
F1, F2 gestation	25.4	53.0	133.7	17.3*	37.5*	116.6*	23.1	46.5*	114.7*	22.7	**45.5***	**119.0***

* $p < 0.05$ significantly different from controls

Through the pre-mating and post-mating periods, food consumption for both F0 and F1 males at 100 mg/kg/day were minimally lower (at most -11%) than corresponding controls (Table 4.11.1.1-3). In

females at 100 mg/kg/day, food consumption was slightly lower than control values during the first 2 weeks of the first gestation (F1A: -20% and then -15%, see Table 4.11.1.1-4). Food consumption in treated females was at least equivalent to control values for the 3rd week of F1A gestation, and for the whole of F1B and F2 gestations and of the three lactation periods.

Table 4.11.1.1-3 : Food consumption (g/animal/day) in F0 and F1 adults – pre-mating and post-mating periods

Sex	**males**				**females**			
Dose level (mg/kg/day)	**0**	**5**	**30**	**100**	**0**	**5**	**30**	**100**
F0- pre-mating (day 71)	29.4	29.2	29.1	**27.8**	21.7	22.1	**19.8**	**20.3**
F1- pre-mating (day 85)	30.0	27.1	27.2	**26.7**	23.4	19.0	**18.9**	**19.5**
F0- post F1A mating (day 141)	28.7	29.2	29.4	**27.3**	-	-	-	-
F0- post F1B mating (day 218)	29.9	30.9	30.1	**28.1**	-	-	-	-
F1- post F2 mating (day 148)	30.3	27.4	27.6	**27.5**	-	-	-	-

Table 4.11.1.1-4: Food consumption (g/animal/day) in F0 and F1 females - gestation period

Dose Level (mg/kg)	0			5			30			100		
Weeks of gestation	1	2	3	1	2	3	1	2	3	1	2	3
FO females, F1A gestation	22.0	24.8	27.7	21.4	24.2	26.2	18.9	22.2	25.7	**17.6**	**21.0**	25.9
FO females, F1B gestation	24.6	28.2	27.1	24.0	26.3	27.0	22.0	24.4	26.6	23.2	26.5	27.9
F1 females, F2 gestation	22.1	26.1	27.6	19.8	24.6	25.3	19.9	23.7	23.9	21.7	25.2	28.1

No statistically significant treatment-related effects on mating, conception and gestation indices, sex ratio or gestation length were observed at any dose level for the three F1a, F1b and F2 litters. Yet, male and females conception indices were slightly (at most -14%) and dose-relatedly lower for F1A and F1B matings, among high-dose and sometimes (F1B) mid-dose animals. This was not observed for F2 mating. The absence of statistical significance and the absence of dose-relationship in the F2 mating led to the conclusion that these findings were not biologically relevant.

Table 4.11.1.1-5: Conception indices for treated and control rats

Group		1		2		3		4	
Dose-level (mg/kg/day)		0		5		30		100	
Sex		M	F	M	F	M	F	M	F
F0 adults	F1A mating	**100.0**	100.0	96.3	96.7	96.4	96.7	**89.7**	**89.7**
	F1B mating	**93.3**	96.6	90.0	90.0	**85.7**	**82.8**	**86.2**	**82.8**
F1 adults	F2 mating	**79.3**	76.7	80.0	75.9	88.5	86.7	75.9	75.9

There were no biologically significant differences in pup survival indices, litter size, number of pups born alive or dead, pup body weights at any dose level in the 3 litters. No alterations external or internal were found to be treatment-related at the pup examination at culling. All observations were considered incidental to the administration of metosulam.

The terminal body weights of males given 100 mg/kg/day were lower than controls (significant for F1 males only, Table 4.11.1.1-6). These differences were consistent with the in life body weight data and appeared to be related to voluntary feed restriction.

In general, the absolute and relative kidney weights were lower than controls in all high-dose groups (F0, F1 males and females: up to -12% relative weight). This was associated with histopathologic renal changes and therefore considered to be treatment-related.

Table 4.11.1.1-6: Mean terminal body weights (g) and kidney weights in F0 and F1 adults

Dose level (mg/kg/day)		**0**		**5**		**30**		**100**	
Sex		**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
F0	Terminal BW (g)	597.4	311.1	589.8	311.5	577.9	314.3	**575.9**	311.2
	Absolute kidney weight (g)	3.938	2.285	3.968	2.289	3.734	2.152	**3.415***	**2.015***
	Relative kidney weight (%)	0.663	0.737	0.675	0.738	0.649	0.686*	**0.596***	**0.651***
F1	Terminal BW (g)	577.0	307.5	534.8	283.9	551.4	287.8	**528.0***	295.0
	Absolute kidney weight(g)	3.678	2.249	3.442*	2.152	3.493	2.056*	**3.178***	**1.909***
	Relative kidney weight (%)	0.641	0.736	0.647	0.760	0.637	0.718	0.607	**0.650***

* $p < 0.05$ significantly different from controls

No gross observations attributable to administration of the test compound were found in any adult or weanling at gross necropsy.

Histopathologic alterations attributable to administration of metosulam were confined to kidney tissue of F0 or F1 adult rats administered 100 mg/kg/day. In F0 male rats these consisted of slight nuclear pleomorphism (30/30 males) and slight hypertrophy of tubule epithelial cells (22/30 males). In F1, 23/30 males demonstrated slight nuclear pleomorphism, 5/30 males demonstrated very slight nuclear pleomorphism, and 7/30 males demonstrated hypertrophy of tubule epithelial cells. In F0, 24/30 females had very slight nuclear pleomorphism; in F1 25/30 females demonstrated very slight nuclear pleomorphism. Nuclear pleomorphism consisted of a divergence from the normal nuclear size and appearance. Those rats with pleomorphism demonstrated anisokaryosis (variable size of nuclei), peripheral condensation of nuclear chromatin, and prominent nucleoli. All other lesions were considered incidental and not treatment-related.

Table 6.6.1-7: Histopathological findings in the kidneys of F0 and F1 adults

Dose level (mg/kg/day)			**0**		**5**		**30**		**100**	
Sex			**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
F0	Nuclear pleomorphism	very slight	0	0	0	0	0	0	**0**	**24**
		slight	0	0	0	0	0	0	**30**	0
	Hypertrophy proximal tubules		0	0	0	0	0	0	**22**	0
F1	Nuclear pleomorphism	very slight	0	0	0	0	0	0	**5**	**25**
		slight	0	0	0	0	0	0	**23**	0
	Hypertrophy proximal tubules		0	0	0	0	0	0	**7**	0

Conclusion

The dietary administration of Metosulam to adult male and female Sprague-Dawley rats throughout 2 generations at dose-levels up to 100 mg/kg/day produced systemic toxicity on adults, but no relevant effect on reproduction neither in the investigated litter parameters :

- Slightly lower food intake and/or body weight gain (up to -12%) in males and/or females treated at 100 mg/kg/day during pre-mating, post-mating, gestation and lactation. Reduced appetence was probably in cause.
- Histopathologic kidney alterations (nuclear pleomorphism and hypertrophy of proximal tubules) and decreased kidney weights (absolute and relative) in the F0 and F1 adults treated at 100 mg/kg/day.

The NOAEL for parental toxicity in this study was 30 mg/kg/day for both sexes.

Conception rates were slightly lower at 30 and 100 mg/kg/day in the F1 mating group but the absence of statistical significance and the absence of dose-relationship in the F2 mating group led to the conclusion that these findings were not biologically relevant. Other indices of reproductive performance (gestation success and gestation length) were unaffected. The male/female sex ratio, gestation survival and survival through day 21 postpartum, litter size and average pup body weight were also unaffected by Metosulam administration at the dose levels tested. The NOAEL for reproductive and neonatal effects was 100 mg/kg/day.

4.11.1.2 Human information

No reported incidents of adverse effects during the manufacture or formulation of metosulam.

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

The dietary administration of metosulam in the diet to pregnant Sprague Dawley rats at dose-levels of 0, 100, 500 or 1000 mg/kg/day, from gestation days 6 to 21 included, induced slight maternal toxicity consisting in slightly lower body weight gain at 500 and 1000 mg/kg/day. No significant treatment-related adverse embryo-fetal effects were observed at any dose level tested.

The dietary administration of metosulam in the diet to pregnant rabbits at dose-levels of 0, 30, 100 or 300 mg/kg/day from gestation days 7 to 19 included, produced mortality in 3/24 rabbits given 300 mg/kg/day as well as necrosis and inflammation of gallbladders, degenerative kidney lesions of moderate severity, and very slight to moderate renal tubule cell necrosis in some rabbits given 100 or 300 mg/kg/day. No significant adverse embryo-fetal effects were observed at any dose level tested.

4.11.2.2 Human information

No reported incidents of adverse effects during the manufacture or formulation of metosulam.

4.11.1 Other relevant information

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4.11.4 Summary and discussion of reproductive toxicity

The dietary administration of Metosulam to adult male and female Sprague-Dawley rats throughout 2 generations at dose-levels up to 100 mg/kg/day produced systemic toxicity on adults (slightly lower food intake and/or body weight gain, histopathologic kidney alterations and decreased kidney weights), but no relevant effect on reproduction neither in the investigated litter parameters. Conception rates were slightly lower at 30 and 100 mg/kg/day in the F1 mating group but the absence of statistical significance and the absence of dose-relationship in the F2 mating group led to the conclusion that these findings were not biologically relevant. Other indices of reproductive performance (gestation success and gestation length)

were unaffected. The male/female sex ratio, gestation survival and survival through day 21 postpartum, litter size and average pup body weight were also unaffected by Metosulam administration at the dose levels tested.

The dietary administration of Metosulam in the diet to pregnant Sprague Dawley rats at dose-levels of 0, 100, 500 or 1000 mg/kg/day, from gestation days 6 to 21 included, induced slight maternal toxicity but no significant treatment-related adverse embryo-fetal effects were observed at any dose level tested.

The administration of Metosulam by gavage to pregnant rabbits at dose-levels of 0, 30, 100 or 300 mg/kg/day from gestation days 7 to 19 included, produced maternal toxicity (mortality in 3/24 rabbits given 300 mg/kg/day as well as necrosis and inflammation of gallbladders, degenerative kidney lesions of moderate severity, and very slight to moderate renal tubule cell necrosis in some rabbits given 100 or 300 mg/kg/day), but no significant adverse embryo-fetal effects were observed at any dose level tested.

4.11.2 Comparison with criteria

The CLP criteria for classification of substances for skin reproductive toxicity (category 2), are as follow:
"There is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification.
Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.

Considering that no specific effect is reported in human and that no adverse effect were observed on sexual function and fertility, or on development in the experimental studies available, no classification is required for metosulam under either Directive 67/548/EEC or the CLP Regulation."

4.11.6 Conclusions on classification and labelling

No classification is required for metosulam under either Directive 67/548/EEC or the CLP Regulation.

4.12 Other effects

No other effects.

4.12.1 Non-human information

4.12.1.1 *Neurotoxicity*

No neurotoxicity data have been reported for Metosulam.

4.12.1.2 *Immunotoxicity*

No immunotoxicity data have been reported for Metosulam.

4.12.1.3 *Specific investigations: other studies*

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4.12.1.4 *Human information*

No reported incidents of adverse effects during the manufacture or formulation of metosulam.

4.12.2 Summary and discussion

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4.12.3 Comparison with criteria

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4.12.4 Conclusions on classification and labelling

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5 ENVIRONMENTAL HAZARD ASSESSMENT

The environmental fate properties assessment for metosulam is based on the Draft Assessment Report (EC, 2006), the Addendum to the Draft Assessment Report (EC, 2010) and the conclusion on the peer review of the pesticide risk assessment of the active substance metosulam (EFSA 2010).

All the studies on the fate and behaviour of metosulam in the environment were performed on GLP and according to UBA, EPA or OECD guidelines. Then their reliability factor was stated to 1.

5.1 Degradation

Table 19. Summary of relevant information on biodegradation

Method	Results	Remarks	Reference
OECD 301 D	Not readily biodegradable	None	Jenkins, W. R. (1991)

5.1.1 Stability

5.1.1.1 *Hydrolysis*

A hydrolysis study of metosulam is available.

Yon, D. A. (1990):

This study is performed according to EPA guidelines and is GLP.
Hydrolysis of metosulam was investigated in sterile 0.01 M aqueous buffer solutions which were adjusted to pH 5, pH 7 and pH 9.

For each pH value, test solutions of A-metosulam (aniline labeled, specific activity of 41.9 µCi/mg, radiochemical purity > 98%) and T-metosulam (triazole labeled, specific activity 53.4 µCi/mg, radiochemical purity > 98%) were prepared at a concentration of 2 mg a.s./L by adding 0.25 mL acetone solution containing the test substance to 25 mL buffer. In addition, for each pH, one serie of samples was spiked with unlabelled metosulam for checking the pH at weekly intervals.

Samples were maintained in the dark at 25°C for 30 days and taken at day 0, 1, 3, 7, 14, 20 and 30 after application.

Total radioactivity was determined by LSC. Identification of metosulam and possible degradates was performed using TLC and by means of mass spectrometry of sample extracts. Quantification was done by TLC with radio-TLC scanner.

The pH value remained relatively constant in all buffer solutions except at pH 9, where a slight decline from pH 8.9 to pH 8.5 was observed during the course of the study.

The total radioactivity determined by LSC ranged from 94.4 to 107.9% AR (applied radioactivity) without any tendency of decline and an overall mean of 100.8% AR, indicating that no relevant volatile radioactivity was formed during the study.

Only the parent compound was detected by TLC at all sampling dates and pH values. The concentrations of metosulam remained in the range of 94.5 to 99.3% AR without any tendency of decline. Besides the peak of the parent compound, only very low amounts of scattering background radioactivity were detected, but no further peaks were found.

No hydrolysis took place at pH 5, 7 and 9 at 25°C in the dark during the 30-day incubation period. Metosulam is therefore regarded as hydrolytically stable at pH 5-9 and 25°C (DT_{50} > 1 year).

5.1.1.2 *Photolysis*

In water

Two studies of the photolysis of metosulam in water are available.

Hellpointner, E. (2002):

This study is performed according to UBA guidelines and is GLP.
The quantum yield of direct photodegradation of metosulam was determined in polychromatic light. In two photodegradation experiments, unlabelled metosulam at approximately 5.5 mg a.s./L water was irradiated for 500 minutes at room temperature (25°C) in a merry-go-round apparatus (TQ Hg-lamp with Duran 50 filter). The concentration of the test compound was determined by reversed-phase HPLC and evaluation of the respective UV-signal by the external standard method.

The UV-spectrum of metosulam was measured in a solution of 11 mg a.s./L in pure water and aqueous buffer solutions at pH 4, 7 and 9 (+ 0.6% acetonitrile).

The quantum yield was calculated from the kinetic results of both photodegradation experiments and from UV absorption data by using the QUANT-software.

The UV-VIS absorption spectra of metosulam in aqueous solutions show slight differences depending on the pH value and the use of pure water or buffer solution.

In pure water metosulam absorption showed a maximum at 211 nm and a shoulder in the range of 240 to 270 nm. The absorption extended rather weak but extremely far into the range of wavelengths relevant for the environment and ended at 768 nm.

In buffer solution:

- at pH 4 the absorption maximum was observed at 206 nm. A shoulder in the range of 240 to 270 nm was found. End of absorption was measured already at 396 nm.
- at pH 7 metosulam showed highest absorption at 212 nm and shoulder from 240 to 270 nm. As at pH 4, absorption ended at 396 nm.
- at pH 9 the absorption maximum was at 212 nm and the shoulder was observed in the range of 240 to 270 nm. The end of the absorption was measured at 383 nm.

The two degradation lines obtained in the exposure tests in solutions of the test compound in pure water showed a moderate, but significant degradation of about 35% after an irradiation period of 500 min. This is in good agreement with the absorption spectrum in pure water, extended far to high wavelengths. The mean experimental half-life calculated from both degradation lines was 35.8 hours.

From the UV-absorption data and the degradation kinetics, the quantum yield in pure water was calculated to be $\Phi = 0.00022$.

The quantum yield and UV-absorption data in aqueous solution were used to estimate the environmental half-life of metosulam in water by two different simulation models. The estimates based on the GC-SOLAR model resulted in environmental direct photolysis half-lives of 2.2 days in summer at 50° lattitude (comparable to German conditions). Calculations based on the model of Frank & Klöpffer resulted in environmental direct photolysis half-lives of 2.5 to 13 days in summer (July) at 50° latitude and 2 to 66 days for the period of main use (February to October).

In pure water, photodegradation in water 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 > 396 nm wavelengths was observed.

Hawkins, D. R.; Mayo, B. C.; Pollard, A. D.; Hughes, C. B. (1992):
This study is performed according to EPA guidelines and is GLP.
Aqueous photolysis of metosulam was studied in sterile 0.01 M aqueous buffer solution adjusted to pH 7 using two radiolabels. Test solutions were prepared separately with A-metosulam (specific activity 53.4 µCi/mg, radiochemical purity > 96%), and T-metosulam (specific activity 64.4 µCi/mg, radiochemical purity > 95%) at an initial concentration of 2 mg a.s./L by adding small aliquots of concentrated solution of test compound in ethanol-buffer (2:1) to buffer solution. The final ethanol concentration in the test vessels was 0.8% ethanol. Test samples were prepared with both labels separately, dark control samples were prepared with T-metosulam only.
Sterile samples were continuously irradiated in borosilicate glass vessels with a Xenon lamp producing artificial sunlight. The lamp was equipped with a filter cutting off all irradiation below 290 nm. Light intensity of the lamp was measured and compared to natural sunlight determined at the testing facility in Huntington, United Kingdom. Samples were irradiated for 190 hours which was equivalent to 36 natural sunlight days in summer (mid of June) at Huntington, UK (latitude 52° 12' N, longitude 0° 15' W). The temperature of the irradiated test vessels was maintained at 25±1°C. Control samples were maintained in the dark at 24±1°C.

Irradiated samples of each radiolabel, as well as duplicate dark control samples, were taken for analysis at 0, 10, 33, 77, 120 and 190 hours, corresponding to 0, 2, 6, 14, 23, and 36 natural sunlight days.

Determination and identification of the parent compound and degradates was performed using normal phase and reversed phase TLC using solvents, radioactivity and UV detector. Total radioactivity in the testing solutions was determined by LSC. Radioactive volatiles were trapped in ethylene glycol and 1 M NaOH and also determined by LSC.

For each sampling date, microbiological activity in the samples was determined to confirm sterility.

All samples of irradiated solutions were shown to have no microbiological activity during the course of the study. Total recovery of radioactivity in all test solutions after incubation for 0-190 hours ranged from 100 to 107% AR, while volatiles accounted for <1% AR.

During the first intervals (0-10 or 33 hours), metosulam concentrations ranged from 95 to 97% AR (A-label) and from 93 to 98% AR (T-label). Later, metosulam decreased slightly to 83 or 77% AR, respectively, at the end of the incubation period. Four minor metabolites were detected in maximum amounts of 2-6% AR. They were characterised by TLC, but not identified. Further unallocated fractions were detected by TLC, each of them amounting to <8% AR.

In the dark control metosulam was stable. The mean concentration of T-metosulam remained at 95% AR during the 190 hours.

The half-life for direct photodegradation of metosulam was calculated by linear regression of the mean values of both labels to be 31.1 days (r^2 = 0.93). This would be equivalent to an environmental half-life of 140 summer days in Huntington, UK.

Direct photodegradation of metosulam in aqueous buffer solution at pH 7 was low with an environmental half-life of 140 summer days in the UK. This low degradation of metosulam is in agreement with the previous study, where no absorption at wavelengths > 396 nm was observed in buffered solutions.

No transformation products of metosulam were observed in significant amounts, since the sum of all unallocated fractions was less than 8% of the applied radioactivity.

On soil

After application of A-metosulam to a silt soil at the application rate of ca 30 g a.s./ha, the degradation was slightly faster under irradiation. The degradation half-lifes DT_{50} were 18.3 days under irradiation, and 42.3 days in the dark, both values exceeding the 10-day duration of the study. Mineralization was low, reaching 2.9% AR of $^{14}CO_2$ in irradiated samples 10 DAT (Day After Treatment). Bound residues increased to 34.3% AR in irradiated soils and 18.4% AR in dark controls 10 DAT. Metosulam was the only compound detected in significant amounts in soil extracts. Beside metosulam, 1.1% of other extractable compounds were found in irradiated soil extracts.

5.1.2 Biodegradation

5.1.2.1 *Biodegradation estimation*

No data.

5.1.2.2 *Screening tests*

A study on the ready biodegration of metosulam is available.

Jenkins, W. R. (1991):

This study is performed according to OECD 301 D guideline and is GLP.
For determination of the Chemical Oxygen Demand (COD) of metosulam, a stock solution of 100 mg a.s./L in pure water was prepared. Aliquots of this solution (2 mL) were mixed with digestion reagent (containing potassium dichromate, sulphuric acid and mercuric sulphate) in reaction vials and boiled under reflux at 150°C for 2 hours. In addition, triplicate blank controls containing pure water instead of stock solution and triplicate control standard containing potassium hydrogen phthalate instead of metosulam were prepared. The

vials were allowed to cool to room temperature and the increase of Cr(III) was determined spectrophotometrically at wavelengths of 420 nm.

Bacterial inhibition was tested using the Closed Bottle Test. Five groups of each four bottles were filled with Mineral Salt Medium (MSM) plus bacterial inoculum, and incubated with the test substance (aqueous suspension of metosulam) and/or sodium benzoate. Four bottles were filled only with MSM, and four others with MSM and inoculum and served as controls. The final concentrations of metosulam in the test vessels were 2 and 10 mg/L. Bottles were incubated for five days in the dark at 20°C. Temperature and dissolved oxygen as well as pH were measured in duplicate samples of each group at the start and at the end of the incubation period using an YSI dissolved oxygen meter.

In the Modified Sturm Test activated sludge from a sewage treatment plant was prepared by homogeneisation and filtration. The filtrate was used as bacterial inoculum. Four test vessels were filled each with MSM and the bacterial inoculum at a concentration of 1%. After aeration with CO_2-free air overnight, sodium benzoate or aqueous dispersions of the test substance (metosulam) were added. The final volume in each vessel was 3 litres.

Vessel 1:	MSM + bacterial inoculum
Vessel 2:	MSM + bacterial inoculum + sodium benzoate (20 mg/L)
Vessel 3:	MSM + bacterial inoculum + metosulam (10 mg/L)
Vessel 4:	MSM + bacterial inoculum + metosulam (20 mg/L)

Samples were continuously flushed with CO_2-free air for 28 days. Concentrations of CO_2 formed in the test vessels and captured in $Ba(OH)_2$ solution were determined by titration of aliquots of the $Ba(OH)_2$ solution with 0.05N HCl, using phenolphtalein indicator.

The COD of metosulam was determined to be 118 mg O_2/mg a.s. This value is higher than the theoretical value assuming reduction of nitrogen to ammonia ($ThOD_{ammonia}$ = 0.96 mg O_2/mg a.s.), and is lower than the $ThOD_{nitrogen}$(1.53 mg O_2/mg a.s.) assuming oxidation to nitrite. This indicates that under test conditions, the carbon content of metosulam was completely oxidized, and nitrogen was partly oxidized.

Oxygen was not consumed in bottles containing the test substance alone, while oxygen consumption in bottles containing sodium benzoate (66% of ThOD) proved that the inoculum was viable under the test conditions. Degradation of benzoate was not essentially affected by presence of metosulam (51% and 54% of the ThOD of benzoate). This indicates that metosulam was not readily biodegradable under the test conditions and that metosulam at the tested concentrations (2 and 10 mg a.s./L) did not inhibit bacterial activity.

In the Modified Sturm Test, cumulative CO_2 production of the blank control was found to be 22.6 mg CO_2 after 28 days which is within the acceptable limit (<50 mg CO_2). The degradation of sodium benzoate was rapid and achieved 63% of its theoretical CO_2 production after 9 days and 78% after 28 days. CO_2 production by mixtures 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 TCO_2. These results indicate that metosulam is not readily biodegradable in the Closed Bottle Test or the Modified Sturm Test.

Metosulam is not ready biodegradable in the Closed Bottle Test or the Modified Sturm Test. At the tested concentrations (2 and 10 mg a.s./l) metosulam did not inhibit bacterial activity.

5.1.2.3 *Simulation tests*

Water

In biologically active water-sediment systems, metosulam has a half-life of 8 days in the water phase as well as in sediment. Degradation leads to formation of three major metabolites: M01, M02 and M04. Maximum amounts of metabolites in water and sediment phases were 17.4% AR 60 DAT and 15.7% AR 120 DAT for

M01, 17.2% AR 14 DAT and 17.8% AR 14 DAT for M02, and 15.6% AR 14 DAT and 4.2% AR 14 DAT for M04, respectively.

Bound residues accounted for 58.7 to 67.5% AR 120 DAT. Mineralization accounted for a maximum of 3.6% AR 120 DAT.

In the total system, the maximal DT50 for metosulam, the metabolites 7-OH-metosulam, 5,7-OH-metosulam and DCM-ATSA-metosulam are respectively 8.2 days, 22.4 days, 14.1 days and 120 days. In the total system, the maximum DT90 for metosulam is 27.1 days. In the sediment and water phase, the maximal DT50 of metosulam are 31.0 days and 7.5 days, respectively.

Soil

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 at 20°C ranged from 4.9 to 43.4 days (all derived with SFO model except the longest half-life derived from bi-phasic kinetic) at 20°C and 40% MWHC (Maximum Water Holding Capacity), with a geometric mean of 10.6 days. The calculated geometric mean degradation half-life after extrapolation at 10°C is 27.3 days.

The metabolite M02 is formed directly from the parent compound by demethylation of the methoxy group at the position 7 of the triazolopyrimidine ring. M01 is formed from M02 by cleavage of the pyrimidine ring. M01 and M02 were rapidly degraded in soils under aerobic conditions at 20°C and 40% MWHC, with a geometric mean DT50 of 54.9 days (derived from bi-phasic kinetic) and 2.2 days (derived from bi-phasic kinetic for two soils of four) respectively.

Under anaerobic conditions, slow degradation of the parent compound was observed, with very low amounts of metabolites (< 3.5%). DT50 of metosulam was higher than 1 year.

After application of metosulam to bare field soils, metosulam was almost exclusively distributed in the 10-cm superficial layer of soil. First-order kinetic dissipation rate allowed to calculate mean DT50 of 31.9 days (range 20-47 days) and a mean DT90 of 104.2 days (range 90-146 days). These DT50 are in good agreement with the previous DT50 obtained in laboratory conditions.

Based on these findings the route of degradation of metosulam in soil can be described as follows: initial step is the microbial demethylation of the methoxy-groups at the triazolopyrimidine ring, preferably in position 7, resulting in M02. In the following step cleavage of the pyrimidine ring to the aminotriazolo ring and formation of M01 takes place. Further degradation of M01 leads to incorporation of fragments of metosulam into the soil matrix and thus formation of high amounts obound residues. To a lower but significant extent, mineralisation and formation of carbon dioxide takes place.

5.1.3 Summary and discussion of degradation

5.1.3.1 *Fate and behaviour in water*

Metosulam was found to be hydrolytically stable at pH 5, 7 and 9 at 25°C.

From the UV-absorption data and the degradation kinetics, the quantum yield in pure water was calculated to be $\Phi = 0.00022$. In pure water, photodegradation in water 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 > 396 nm wavelengths was observed.

Photolysis in buffered solution at pH 7 took place at a low extent, even if the quantum yield indicates that photolysis could be a reasonable route for dissipation of metosulam in environment. The environmental half-

live of 140 days (equivalent in UK) of metosulam in aqueous buffered solution suggests that aqueous photolysis plays a secondary role for dissipation of metosulam from aquatic systems under environmental conditions.

Metosulam is not ready biodegradable in the Closed Bottle Test or the Modified Sturm Test. At the tested concentrations metosulam did not inhibit bacterial activity.

In biologically active water-sediment systems, the maximal DT50 in the total system for metosulam, the metabolites 7-OH-metosulam, 5,7-OH-metosulam and DCM-ATSA-metosulam are respectively 8.2 days, 22.4 days, 14.1 days and 120 days. In the total system, the maximum DT90 for metosulam is 27.1 days. In the sediment and water phase, the maximal DT50 of metosulam are 31.0 days and 7.5 days, respectively.

Since the maximal DT50 and DT90 in the total system are 8.2 and 27.1 days, respectively, more 70 % of metosulam would be degraded in 28 days.
In addition, according to the 2nd ATP to the regulation (EC) 1272/2008, the degradation products could have to be considered in the assessment of rapid degradability. Considering metosulam is significantly more toxic for algae and aquatic plants, the aquatic toxicity data for degradation products available only for algae and aquatic plants are considered sufficient to fully address their hazard profile for aquatic environment. Since the degradation products do not fulfill the criteria for classification as hazardous to the aquatic environment (ErC50 > 1 mg/L and NOEL > 1 mg/L), primary biodegradation of metosulam is considered sufficient in the assessment of rapid degradability of this substance.
Therefore, metosulam is considered as rapidly degradable according to the CLP regulation.

5.1.3.2 *Fate and behaviour on soil*

After application of metosulam to bare field soils, metosulam was almost exclusively distributed in the 10-cm superficial layer of soil. First-order kinetic dissipation rate allowed to calculate mean DT50 of 31.9 days (range 20-47 days) and a mean DT90 of 104.2 days (range 90-146 days). These DT50 are in good agreement with the previous DT50 obtained in laboratory conditions.

Based on these findings the route of degradation of metosulam in soil can be described as follows: initial step is the microbial demethylation of the methoxy-groups at the triazolopyrimidine ring, preferably in position 7, resulting in M02. In the following step cleavage of the pyrimidine ring to the aminotriazolo ring and formation of M01 takes place. Further degradation of M01 leads to incorporation of fragments of metosulam into the soil matrix and thus formation of high amounts obound residues. To a lower but significant extent, mineralisation and formation of carbon dioxide takes place.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

Sorption properties of metosulam in soil were investigated in several batch equilibrium tests as well as column leaching experiments with aged and non-aged soil. The Freundlich adsorption coefficients K_{FOC} determined in batch equilibrium tests performed with 4 soils ranged from 51.5 to 264.7 (mean K_{FOC} = 166.1). Adsorption of metosulam is predominantly influenced by the organic carbon content of the soil. Significant pH dependence of adsorption is not expected in a normal range of agricultural soil pH.

5.2.2 Volatilisation

Based on the low vapour pressure (1×10^{-12} Pa at 25°C), metosulam is not considered as a volatile substance.

5.2.3 Distribution modelling

Not relevant for this report.

5.3 Aquatic Bioaccumulation

5.3.1 Aquatic bioaccumulation

Based on its log Kow values of 1.8 (pH 4 at 20°C), 0.2 (pH 7 at 20°C) and -1.1 (pH 9 at 20°C), the bioaccumulation potential of metosulam is predicted to be low.

However, based on incorrect values of log Pow determined initially (2.12 at pH = 5, 2.46 at pH = 7 and 3.1 at pH = 9), a study of bioconcentration in aquatic organisms has been performed by Hawkins *et al.* (1992). In this study performed according the OECD 305 guideline (reliability = 1), there was no evidence of bioaccumulation of metosulam in fish tissues after exposure to the active substance.

5.3.2 Summary and discussion of aquatic bioaccumulation

Based on a log Kow value of 0.2 (pH 7 at 20°C) and in absence of bioconcentration of metosulam in the experimental study (Hawkins *et al.*, 1992), the cut-off value of BCF ≥ 500 (and log Kow ≥ 4) set out in the CLP Regulation is not exceeded.

5.4 Aquatic toxicity

Only reliable and validated ecotoxicity tests accepted for risk assessment from Draft Assessment Reports were used. Then, the reliability factor would be indicated in the summary only when different of 1. The reliability factors of the aquatic toxicity studies are reported in the Table 20, which summarised the available data on the toxicity for aquatic organisms. Aquatic plants are the most sensitive species (see section "5.4.3. Algae and aquatic plants").

5.4.1 Fish

5.4.1.1 *Short-term toxicity to fish*

Three short-term toxicity studies to fish are available for metosulam.

Dorgerloh, M.; Sommer, H. (2002a):

This test was GLP and performed according to OECD guideline no 203 (1992). The tested species was *Oncorhynchus mykiss* (rainbow trout).
Two groups of 15 rainbow trouts (*Oncorhynchus mykiss*) per replicate (all test levels in duplicates) (mean body length 55 mm, mean body weight 1.8 g) were exposed for 96 hours under static conditions to technical metosulam (batch WP-890227, purity: 98.4 % at nominal concentrations of 0 (controls) and 100 mg/L water.

Test material was mixed into water as homogeneously as possible by ultrasonication. Achieved concentrations were controlled on days 0, 2 and 4. For this analysis, samples from each replicate were taken on days 2 and 4 and filtered (with a pore width of 0.22 µm) to determine actually dissolved metosulam. Additional samples were taken from the same replicate on days 2 and 4 and were mixed for a few minuts in order to determine total concentration of the test item in the vessels.

Observations for mortalities, clinical signs and water characteristics (temperature, pH, oxygen, observable solubility of test substance) were performed 4 hours after treatment start and daily thereafter.

The total and dissolved test material achieved concentrations in water were largely below nominal concentrations at all sampling times. The test substance was observed to be partially soluble in test water, and mean actual concentration of dissolved metosulam was determined to be 29.3 mg/L, i.e.; 29.3% of nominal concentration. The total concentration of metosulam on day 4 ranged from 98 to 102% of the values measured on day 0. The concentrations of dissolved metosulam were not determined on day 0, but were identical on days 2 and 4.

Oxygen concentration, temperature and pH were similar in the different solutions and did not show marked variations along the study. The pH values ranged from 7.1 to 7.2

There were no mortalities. Yet about 23% of fish presented some clinical signs: laid inactive on the bottom of the aquarium, were inactive or displayed abnormally low activity, remained for unusually long periods at the water surface or showed labored respiration.

The 96h static LC_{50} is **higher than** its practical limit of water solubility i.e. **29.3 mg/L** (measured concentration) under the static test conditions.

The no-observed-effect concentration (NOEC) was not determined in the study since sublethal effects were observed at the single tested concentration.

Dill, D. C.; Gorzinski, S. J.; Richardson, C. H.; Potter, R.B. (1990):

This test was GLP and performed according to OECD guideline no 203 (1984). The tested species was *Pimephales promelas* (Fathead Minnows).
Six groups of five Fathead Minnows (*Pimephales promelas*; mean length: 25mm, mean weight: 0.22g) were exposed under static conditions to a nominal concentration of 100 mg/L technical metosulam (batch AGR 275252, purity 98.9%) for 96-hours at 22°C. There were six control groups: 3 without any treatment and 3 others with the carrier solvent acetone (0.5 mL/L).

The test substance was dissolved in acetone and then diluted in industrial water. Achieved concentrations were measured at initiation and at termination of the test using a validated HPLC-UV detection method at 254 nm and with a LOQ of 1.50 µg/L.

Observations for mortalities and clinical signs were performed daily.

Undissolved test substance was reported to be present in water with metosulam. On day 0 as on day 4, actual concentrations were below 80% of nominal value, but they were quite stable with on overall mean concentration of 53.2 mg/L. Oxygen and temperature did not show marked variations along the study. pH ranged from 7.1 to 7.9.

A single mortality occurred in the treated group, while one and two mortalities occurred respectively in water and control group. No clinical signs were reported.

The Fathead Minnows 96h static LC_{50} was **higher than** the limit of solubility in test water in this study (**53.2 mg/L**). The NOEC was 53.2 mg/L (mean achieved concentration).

Faggella, G. A. (1990b):

This test was GLP but no guidelines were mentioned in the report (reliability factor 2). The tested species was *Menidia beryllina* (Tidewater Silverside).

Groups of 30 Tidewater Silverside (*Menidia beryllina*; mean length: 44 mm, mean weight: 0.46 g), were exposed under static conditions to nominal concentrations of 0 (controls) or 100.2 mg/L technical metosulam (batch AGR 275252, purity 98.9%) for 96 hours at 22°C.

Solubility in seawater and 4-day stability were checked. Measurements of achieved concentrations were performed by a validated HPLC-UV detection at 230 nm at 22°C with a LOQ of 0.53 mg/L. Samples were filtered through a 0.45 µm membrane filter before the measurement of actual concentrations.

Observations for mortalities and clinical signs were performed daily.

Solubility in seawater was 128.2 mg/L. The 4-day stability in salt water at 22°C was satisfactory. Achieved concentration for a nominal value of 100.2 mg/L technical metosulam was satisfactory (93.2 mg/L).

Temperature and salinity were checked and did not show marked variations along the study. pH ranged between 7.4 and 8. Oxygen fell to 47% during the first 48h and dissolved oxygen concentrations ranged from 3.3 to 7.1 mg/L. Dissolved oxygen concentration was lower than that recommended in the OECD 203 and EPA 712 guidelines, but both control and metosulam-treated fish did not seem to be affected and this parameter is not considered to not influence the outcome of the study.

There were no mortalities or clinical signs.

The Tidewater Silverside 96h static LC_{50} for technical metosulam was **> 93.2 mg/L**. The NOEC was 93.2 mg/L (achieved concentration).

5.4.1.2 *Long-term toxicity to fish*

Two long-term toxicity studies to fish are available for metosulam.

Douglas, M. T., Bell, G., Macdonald, I. A. (1991a):

This test was GLP and performed according to OECD guideline no 204 (1984). The tested species was *Oncorhynchus mykiss* (rainbow trout).
Six groups of 10 young Rainbow trouts (*Oncorhynchus mykiss;* mean body length 60 mm, mean body weight 3.35 g) were exposed for 21 days under semi-static conditions to nominal technical metosulam (batch AGR 275252, purity: 98.9%) concentrations of 0 (controls), 0.8, 2.5, 8.0, 25 or 80 mg/L at 14ºC.

Stock solutions were prepared overnight using vigorous agitation at test temperature and the media containing the test material were renewed daily.

Solubility and stability were checked. Test material concentrations were controlled daily before and after the renewal of the media. Achieved concentration measurements were performed by HPLC-UV detection at 254 nm with a limit of determination of 0.32 mg/L.

Observations for mortalities, clinical signs and water characteristics (temperature, pH, oxygen) were performed daily.

The solubility was determined to be 78.96 mg/L. The achieved concentrations of the test material in water were between 82-106% of nominal concentrations at all sampling times in fresh and in 24h-expired media so that the stability was satisfactory. Oxygen, water temperature and pH were similar in the different solutions and did not show marked variations along the study.

There were two deaths at the highest test material concentration, on days 16 and 18, and one death in the control group on day 20. Clinical signs were observed at the highest test material concentration: patchy pigmentation, intermittent loss of equilibrium, labored swimming (in the fish which died afterwards). The treatment did not induce any significant body weight variations.

The Rainbow trout 21 day static LC_{50} appears to be higher than 76 mg/L. Considering adverse effects on equilibrium, pigmentation and the two mortalities observed at 76 mg/L, the lowest tested concentration with effect (LOEC) was 76 mg/L, and the highest tested concentration without effect (NOEC) was **24.4 mg/L** (mean measured concentration).

Gries, T. (2002a):

This test was GLP and performed according to OECD guideline no 210 (1992). The tested species was *Pimephales promelas* (Fathead Minnows).
Six groups of 120 Fathead minnow eggs (*Pimephales promelas*, < 24 hours old, 2 replicates of 60 eggs) were exposed under flow-through conditions (flow rate: 50 mL/min) to technical metosulam (batch WP-890227, purity: 98.7 %) at nominal concentrations of 0.625, 1.25, 2.50, 5.00, and 10.0 mg/L water at 25± 1ºC for 32 days. There was a control group without any treatment and a solvent control group. Eggs were laid in incubation cups. The start of exposure was Day 0. After hatching, 40 larvae were randomly selected in each replicate (day 4 after start of the exposure = day 0 post-hatch).

Technical metosulam was diluted in dimethyl formamide (final concentration: 0.2 mL/L) and this mix was injected in the dilution water by a pump system. Test material concentrations were controlled for each solution at the start of the exposure, weekly from alternatively replicates A or B thereafter and at the end of exposure from all test vessels.

Temperature, dissolved oxygen and pH were measured in each aquarium at the start of the exposure, weekly thereafter and at the end of exposure on day 28 post-hatch.

Eggs were examined daily and dead eggs were recorded. After hatching, the number of live, deformed, dead was recorded and the percentage of hatch was calculated as the number of live fry/60 corresponding eggs. Then fry were observed for mortality and sublethal effects.

The achieved concentrations of the test material in water ranged from 85 to 96% of nominal concentrations at all sampling times except for the highest concentration: 44% of nominal value. Therefore, all results are based on the mean measured active substance concentrations. Due to the limited solubility of the test item, the final solvent concentration was increased to 0.2 mL/L instead of 0.1 mL/L. This deviation is considered to have no effect on the interpretation of the results of this study since no adverse effects were observed in the solvent control when compared to the control. Oxygen, water temperature and pH were similar in the different solutions and did not show marked variations along the study.

Time of hatching in treatment exposed eggs was similar to the control groups. The hatching rate was not statistically different in the different groups and post-hatching survival was not reduced in treated fish. Only one replicate of the high-dose group showed brutal mortality but this incident was caused by a technical problem and was not related to the treatment. The notifier considered that this replicate would have had the same survival rate as the other replicate if no incident occurred. However, it is not possible to extrapolate the fate of these larvae, and so the high dose group was not used for the determination of the NOEC by the RMS. The few larval deformities reported were not treatment related; body weight and length were not affected at any concentration.

The NOEC for hatching parameters and larval survival and growth of the Fathead minnow was determined to be **4.24 mg/L** (mean measured concentration) considering the lack of data in the high-dose group due to accidental mortality in one replicate.

5.4.2 Aquatic invertebrates

5.4.2.1 *Short-term toxicity to aquatic invertebrates*

Three short-term toxicity studies to aquatic invertebrates are available for metosulam.

Dorgerloh, M. & Sommer, H. (2002b):

This test was GLP and performed according to OECD guideline no 202 (1984). The tested species was *Daphnia magna*.
Groups of 3 replicates of 10 daphnids (first instars of *Daphnia magna*, ≤ 24h old) were exposed to nominal concentrations of 0, 1.56, 3.13, 6.25, 12.5, 25, 50 and 100 mg/L of technical metosulam (batch WP-890227, purity 98.4 %) for 48 hours under semi-static conditions.
The stock solutions were first prepared in an ultrasonic bath and then stirred for 16 hours. Aliquots were then taken and mixed with culture medium by means of a magnetic stirrer. The concentrations of the test material were measured initially and after 48 hours. Oxygen and pH measurements were scheduled accordingly.

Daphnids were observed for immobilization 24 and 48 hours after introduction into the test solutions.

Achieved concentrations of metosulam were constantly at 99 - 105 % of the nominal value. White powdery precipitates were reported for the two highest doses. Oxygen and pH were within standard ranges in all samples.

No immobilization was observed in controls and only one in the high-dose solution (=3%) during the whole treatment period.

The 48h EC_{50} of metosulam was **greater than 100 mg/L** (nominal concentration).

Faggella, G. A. (1990c):

This test was GLP but no guidelines were mentioned in the report. The tested species was *Paleomonetes pugio* (grass shrimps).
Groups of 30 juvenile grass shrimps (*Paleomonetes pugio*, mean weight: 0.13 g, mean length: 22 mm) per test concentration were exposed for 96 h under static conditions to technical metosulam (batch AGR 275252, purity: 98.9%) at a nominal (mean measured) concentrations of 0 and 100.2 (97.7) mg/L.

Filtered seawater was used for the preparation of test solutions. Solubility and stability of metosulam in salt water were checked.

Observations for mortalities, sublethal signs and water characteristics (temperature, salinity, pH, oxygen, observable solubility of test substance) were performed daily.

Water characteristics were within standard ranges. Solubility of technical metosulam in salt water was 128 mg/L. Achieved test material concentrations were between 93 and 102 % of nominal a.s. concentrations at all sampling times.

There were no mortalities or sublethal effects in the group treated with metosulam.

Grass shrimp LC_{50} could not be determined but was **higher than 100.2 mg/L** (nominal concentration). The NOEC was 100.2 mg/L.

Faggella, G. A. (1990c):

This test was GLP and performed according to EPA 1988 guideline. The tested species was *Crassostrea virginica* (Eastern oysters).
Groups of 20 Eastern oysters (*Crassostrea virginica*, 25 - 50 mm) per test concentration under flow-through conditions were exposed for 96 h to metosulam (batch AGR 275252, purity 98.9%) at nominal concentrations of 0 (control), and 13 , 22, 37, 64, and 100 mg/L.

Unfiltered seawater was used for the preparation of test solutions. The test substance was dissolved in the water by means of a mixer and this stock solution was injected in test vessels by a diluter. Solubility and stability of metosulam in salt water were checked.

Observations for mortalities, sublethal signs and water characteristics (temperature, salinity, pH, oxygen, observable solubility of test substance) were performed daily.

Water characteristics were within standard ranges. Solubility of technical metosulam in salt water was 128 mg/L. Achieved test material concentrations were between 89 and 110% of nominal a.s. concentrations at all sampling times.

The two highest-dose treated group showed a statistically significant decrease of shell growth. There were several mortalities in the groups treated with metosulam at 22 g/L and above, without clear dose-relationship.

Eastern oyster LC_{50} was higher than 100 mg/L. EC_{50} based on shell growth was calculated to be **87.7 mg/L** (nominal concentration). Considering effect of metosulam on shell growth and feces production, the NOEC was 37 mg/L.

5.4.2.2 *Long-term toxicity to aquatic invertebrates*

A single long-term toxicity study to aquatic invertebrates is available for metosulam.

Douglas, M. T.; Bell, G.; Macdonald, I. A. (1991b):

This test was GLP and performed according to OECD guideline no 202 (1984). The tested species was *Daphnia magna*.
Six groups of 40 daphnids (first instars of *Daphnia magna*, ≤ 24h old, 4 replicates of 10 daphnids for each concentration) were treated with technical metosulam (batch AGR 275252, purity: 98.9%) under semi-static conditions, at nominal concentrations of 0 (controls), 0.8, 2.5, 8.0, 25 and 80 mg/L. Mortality and health of adult daphnids were observed daily and number of dead and live young were recorded three times per week, just before each treatment renewal, until study termination on day 21.

Water characteristics (pH, oxygen, temperature) were recorded at least three times per week.

Fresh stock solutions were prepared using agitation overnight at test temperature. Solubility was checked and the concentrations of the test material were measured daily by HPLC-UV at 254 nm with a limit of detection of 0.32 mg/L.

Water characteristics were within standard ranges. Solubility was determined to be 78.96 mg/L. Achieved test material concentrations were between 83-102% of nominal a.s. concentrations at all sampling times, and therefore the stability was satisfactory.

Survival rates of adults at the end of the 21-day period were strongly reduced at the three highest concentrations. There were no sublethal effects at any concentration. Released offspring were first observed on day 7 in all groups. There were no treatment-related aborted eggs or mortalities in the young. Total alive offspring production at the end of the study was reduced in the highest dose groups as the parental mortality rates was higher, but mean alive offspring per surviving adult was similar in all groups. So there was no relevant effect on reproduction.

Taking into account the 17% increase in mortality at 8 mg a.s. /L compared to controls, the parental NOEC is **2.5 mg a.s./L** (nominal concentration). For reproduction, the NOEC is 80 mg a.s. /L.

5.4.3 Algae and aquatic plants

Two toxicity studies to algea are available for metosulam.

Douglas, M. T.; Bell, G.; Macdonald, I. A. (1991c):

This test was GLP and performed according to OECD guideline no 201 (1984). The tested species was *Scenedesmus subspicatus.*
Groups of 3 replicates of *Scenedesmus subspicatus* were treated with technical metosulam (batch AGR 275252, purity: 98.9%) under static conditions, at nominal concentrations of 0.02, 0.04, 0.08, 0.16 and 0.32 mg/L for 96h. A control group (3 replicates at the same cell density) was included. Initial, 72h and 96h cell densities were evaluated for control groups. Growth of all groups was monitored daily by measuring the absorbance of each culture.

The test substance was dissolved in the test medium by stirring overnight. The test material was dispersed into water. The solubility of metosulam in these conditions was checked. The 4-day stability of the active substance was controlled under test conditions both with and without biomass presence. Achieved concentrations of the active substance were controlled before introduction of the alga and at study termination using a validated HPLC-UV method (at 254 nm, limit of determination 0.005 mg/L).

Water pH was recorded at the beginning and the end of the study.

The solubility of metosulam in these conditions was determined to be 80 mg/L. The stability of metosulam, was between 88 and 100% of nominal value in presence or absence of animals. Measured concentrations before alga introduction were within 88-97% of nominal a.s. values. They were within 85-88% of nominal a.s. values at the end of the test. The pH values showed the same variations in all groups except in the high-dose one in which pH was lower at 96h.

Initial cell densities were above the intended value of 10^4 cells/mL and were similar in the different groups. At test termination, biomass inhibition was marked and statistically significant compared to controls, at the three highest metosulam concentrations: 0.08, 0.16 and 0.32 mg/L. In the two highest test concentrations the cells were of reduced size, with ruptured cells present at both 72 and 96 hours at the highest exposure level (0.32 mg/L). No abnormalities were detected in the remaining cultures.

The results are based on nominal concentrations.

The **ErC_{50} (24-48h)** (and corresponding 95% confidence limits) was **0.17 mg**/L (0.15-0.19 mg/L). The **72 h EbC_{50}** (and corresponding 95% confidence limits) was **0.075 mg/L** (0.070-0.081 mg/L). The 96 h EbC_{50} (and corresponding 95% confidence limits) was 0.088 mg/L (0.075-0.10 mg/L). The NOEC at 72 and 96h was 0.02 mg/L in terms of biomass and growth rate.

Jenkins, C. (1999):

This test was GLP and performed according to OECD guideline no 201 (1984), US-EPA-FIFRA 122-2, EEC-C3. The tested species was *Navicula pelliculosa.*
Six groups of freshwater diatom *Navicula pelliculosa* (initially 10^4 cells/mL) were treated with technical metosulam (batch RMM 2215, purity: 99.2%) under static conditions for 120h, at nominal concentration of 100 mg/L. A control group (6 replicates at the same cell density) was included. Cell density was measured for each treatment level at 24-hour intervals. Cell microscopic evaluation was performed at the beginning and the end of the study.

Stock solution was prepared by adding 1 g of test substance into sterile culture medium and then 10 mL of this solution were poured into the definitive medium. The concentrations of the test material were measured before introduction of the alga and at study termination. At each time two samples were taken, one of which was filtered (pore width 0.2 µm) for the determination of actually dissolved concentration.

Water pH and temperature were recorded at the beginning and the end of the study.

The total metosulam concentration was relatively stable during the test, whereas the initial dissolved fraction was only 29% of the value measured at 120 hours, which was close to the total metosulam concentration, suggesting a progressive dilution of the test substance into the medium. The overall mean concentration of dissolved metosulam during the study was 53.6 mg/L. The pH and temperature values were stable and within standard ranges.

Initial cell densities were similar in the different groups. At test termination, there was no biomass or growth rate inhibition and no microscopic abnormalities of the cells were reported at any time in any group.

The **EbC_{50} and ErC_{50}** could not be calculated but seemed to be **higher than 53.6 mg a.s./L** (mean measured concentration).

The NOEC was 53.6 mg a.s./L.

Toxicity data for degradation products

M01 (ATSA): *Scenedesmus subspicatus*: 72-h EbC50 and 72-h ErC50: > 10 mg/L; NOErC: 10 mg/L

M02 (7-OH metosulam): *Scenedesmus subspicatus*: 72-h EbC50 and 72-h ErC50: > 100 mg/L; NOEC: 50 mg/L

M04 (5,7-OH metosulam): *Scenedesmus subspicatus*: 72-h EbC50: 81 mg/L and 72- h ErC50: 101 mg/L; NOEC: 25 mg/L

Single toxicity study to aquatic plants is available for metosulam.

Gries, T., Kolk, van der, J. (2002):

This test was GLP and performed according to OECD guideline no 221 (draft October 2000). The tested species was *Lemna minor*.
Groups of 3 replicates of *Lemna minor* (4 plants in each, initially 3 fronds/plant) were treated with technical metosulam (batch WP-890227, purity: 99.4%) under static conditions from day 0 to day 7, at nominal concentrations of 0, 0.100, 0.320, 1.00, 3.20 and 10.0 µg/L. A control group (3 replicates at the same density) was included. After the exposure period, the plants transferred into untreated medium (recovery phase from day 7 to day 14).

The number of fronds and observation for any abnormalities were assessed on days 0, 3, 5, 7 (during the exposure period) and on days 7, 10, 12 and 14 (during the recovery period). Water pH, temperatures and concentrations of the test material were measured on days 0, 3, 5, 7 (during the exposure period) and on days 7, 10, 12 and 14 (during the recovery period).

Water pH and temperatures were stable and within standard ranges.

Achieved concentrations were between 83-102% of corresponding nominal concentrations on day 0. But on day 7, measured concentrations ranged from 24 to 36 % of corresponding nominal concentration, except for the lowest initial concentration at which measured values were below the LOQ of 0.05 µg/L. The RMS calculated the geometrical mean concentration over the 7-day treatment period.

The doubling time of the control was 2.3 days so it was within the quality criterion of the guideline (<2.5d)

During exposure period, the number of fronds was significantly lower (decrease range respectively from 21 to 82% of control) at the 3 highest concentration means over period (0.556, 1.91 and 6.87 µg/L). At these concentrations, AUC (Area Under the growth Curve) was also reduced and its global inhibition range from

36.99 to 90.48% of control value. The growth rate was inhibited with factors from 16.8 to 81.5% of control value. No other adverse effect was recorded.

After 3 days recovery period, growth rates at concentrations from 0.556 to 1.91 µg/L were similar to those of control group. At the highest concentration, the growth rate was significantly reduced, compared to the control (13%). But after 5 day recovery, all treated groups had a growth rate similar to the control: the effects of metosulam on *Lemna minor* growth are therefore reversible.

Lemna minor were exposed for 7 days to initial nominal metosulam concentrations of 0, 0.100, 0.320, 1.00, 3.20 and 10.0 µg a.s./L under static conditions. Due to the decreases in actual measured concentrations over the time, geometrical mean concentrations were calculated at 0, <0.05, 0.05, 0.15, 0.51, 1.81 and 6.35 µg/L over the exposure period and the results are based on these measured concentrations.

The **7-day ErC_{50}** was calculated to be **0.789 µg/L** (with 95% confidence limits).

The 7-day EbC_{50} was calculated to be 2.30 µg/L.

The 7-day NOEC was 0.15 µg/L for specific growth rate.

Toxicity data for degradation products

M01 (ATSA): *Lemna minor*: 7-day ErC50: > 10 mg/L; NOEC: 10 mg/L

M02 (7-OH metosulam): *Lemna minor*: 7-day EbC50: 16 mg/L and 7-day ErC50: 19 mg/L; NOEC: 3.2 mg/L

M04 (5,7-OH metosulam): *Lemna minor*: 7-day EbC50: 9.39 mg/L and 7-day ErC50: 7.95 mg/L; NOEC: 1.8 mg/L

5.4.4 Other aquatic organisms (including sediment)

No data.

5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

Data are summarised in Table 20 below.

Table 20. Summary of acute and long term toxicity of metosulam to the most sensitive species within different groups of aquatic organisms

Organism	Species	Test conditions	LC_{50}/ EC_{50} (mg/L)	NOEC (mg/L)	GLP (Y/N)	Reliability
Fish	*Oncorhynchus mykiss* (Rainbow trout)	96 h static	> 29.3 (mean measured)	< 29.3	Y	1
	Pimephales promelas (Fathead minnow)	Early life stage, flow-through, 32 days	-	4.24 (mean measured)	Y	1
Invertebrates	*Crassostrea virginica* (eastern oyster)	96 h, flow-through	87.7 (nominal) (Effects on shell growth)	37 (nominal)	Y	1
	Daphnia magna (waterflea)	Growth and reproduction, semi-static, 21 days	-	2.5 (nominal)	Y	1
Algae	*Scenedesmus subspicatus*	Static, 96 h Biomass (72 h): Growth rate (24-48 h):	 0.075 (nominal) 0.17 (nominal)	 0.02 (nominal) 0.02 (nominal)	Y	1
	Navicula pelliculosa	Static, 120 h Biomass: Growth rate:	 > 53.6 (mean measured) > 53.6 (mean measured)	 53.6 (mean measured) 53.6 (mean measured)	Y	1
Aquatic plants	*Lemna minor*	Static, 7 days Biomass Growth rate	 0.0023 (mean measured) 0.000789 (mean measured)	 - 0.00015 (mean measured)	Y	1

In toxicity studies for algae and aquatic plants EC50s at concentrations ≤ 1 mg/L were observed. In addition, metosulam is not readily biodegradable although it is unlikely for the substance to bioaccumulate in aquatic organisms (no evidence of bioaccumulation in fish tissues and log Kow < 3). As a consequence and according to the CLP Regulation, due to its acute effect on algae/aquatic plants at a concentration ≤ 1 mg/L and due to its low degradability, metosulam should be classified as R50/53 (Aquatic Acute 1 – Aquatic Chronic 1).

Based on the toxicity data for *Lemna minor* (ErC50 = 0.000789 mg/L) an M-factor of 1000 is proposed. The same approach was applied to determine specific concentration limits according to Directive 67/548/EEC:

Concentration Classification

C≥0.025% N; R50/53

0.0025%≤C<0.025% N; R51/53

0.00025%≤C<0.0025% R52/53

where C is the concentration of metosulam in the preparation (expressed as weight/weight percentage).

Here is the classification proposal for chronic according the 2nd ATP to the regulation (EC) 1272/2008. The lowest chronic toxicity value was the NOEC = **0.00015** mg/L (Gries, T., Kolk, van der, J. (2002)), determined with *Lemna minor*. As the NOEC-value is between 0.0001 and 0.001 mg/L and metosulam is considered as rapidly degradable, classification as **Aquatic Chronic 1 H410 'Very toxic to aquatic life with long lasting effects'** with a **M-factor of 10** according to Regulation EC 1272/2008 will be proposed

5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

Proposed classification based on Directive 67/548/EEC criteria:

N; R50-53

Proposed specific concentration limits (if any):

Proposed classification of mixtures		
N; R50-53	N; R51-53	R52-53
C ≥ 0.025%	0.025% > C ≥ 0.0025%	0.0025% > C ≥ 0.00025%

The concentration limits are expressed as weight/weight percentage.

Proposed classification based on CLP criteria:

Aquatic Acute 1 – H400

M-factor: 1000

Aquatic Chronic 1 – H410

M-factor: 10

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	OECD 203, GLP	static	96 h LC_{50}: > 29.3 (measured

mykiss			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.

Additional key elements

Ready biodegradability

Concerning the OECD 301D test it is found that the test duration is only 5 days in contradiction of 28 days mentioned in the guideline (EFSA DAR Volume 3, Annex B, B.8 attached to the IUCLID file). Because the validity criteria of the OECD 301D guideline test are not met, the RAC decided not to use the result of this test when evaluating the ready/rapid degradability of the substance.

Aquatic toxicity

The test substance concentration in the *L. minor* test was only measured on day 0 and on day 7 according to the EFSA DAR (Volume 3, Annex B, B.9). On day 0 the concentrations were between 83-102 % of the corresponding the nominal concentrations. On day 7, measured concentration ranged only from 24 to 36 % of nominal concentrations. According to the DS geometric mean concentrations were calculated over the exposure period. However, the EFSA DAR states that the values are time weighted average concentrations (TWA). According to the OECD Guidance on Aquatic Toxicity Testing of Difficult Substances and Mixtures geometric mean concentration would be preferable for static tests where concentrations do not remain within 80-120% of nominal. These are, however, not available and the reported values are used in the evaluation by RAC.

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).

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8 ANNEXES

See appendix 1 for confidential data