

ANNEX B

Metam-sodium

Updated DAR Re-submission August 2010

B.6 Toxicology and metabolism

Preliminary remark:

At the occasion of the re-submission of the EU-dossier of Metam under regulation Reg 33/2008, the DAR was revisited completely. The text was amended and updated with the agreed critical endpoints and reference doses agreed upon during the previous Peer-Review and with the conclusions in the EFSA-report. The changes which were presented in the DAR Addendum of June 2008 and of July 2008 were taken over.

For the sake of transparency, and in order to obtain a consolidated DAR for subsequent use during national authorisation procedures, all amended parts were presented with yellow overlay in this revised DAR. When the conclusions have been peer reviewed, it is preceded with the mention: "Already Peer Reviewed, EFSA 2008" or "Calculation revised after Peer-Review and based upon EFSA conclusions, EFSA, 2008".

The only new data pertain the evaluation of a newly submitted greenhouse (tunnel) trial in order to assess the exposure of operators, workers, bystanders and residents to MITC, for this agricultural intended use.

This study was submitted in an attempt to demonstrate a safe use of Metam application by drip irrigation (1% dilution). For the use of undiluted Metam in open field (soil injection), a safe use was already demonstrated and agreed upon at the EU-level.

Already Peer Reviewed, EFSA 2008

An overview of the batches, used in the various toxicological assays on the a.i. is given below:

Table B.6.1, Batches used during the toxicity studies with Metam-sodium or Metam-potassium

Type of test Test species Used doses	Test substance (source) Batch number Purity	References
Acute oral, rat	Metam-sodium (UCB) B n° not reported 533 g/L	Baldrick and Healing, 1991a
Acute dermal, rat	Metam-sodium (UCB) B n° not reported 533 g/L	Baldrick and Healing, 1991b
Acute inhalation, rat whole body	Metam-sodium (UCB) B n° not reported 533 g/L	Jackson and Hardy, 1992
Acute inhalation, rat head and nose	Metam-sodium (BASF, Metam-fluid) B n° ZH 130585 42.2%	Gelbke and Klimisch, 1986
Acute skin irritation, rabbit	Metam-sodium (UCB) B n° not reported 533 g/L	Ligget and McRae, 1991a
Acute eye irritation, rabbit	Metam-sodium (UCB) B n° not reported 533 g/L	Ligget and McRae, 1991b
Skin sensitization- M&K test	Metam-sodium (UCB) B n° not reported 533 g/L	Parcell and Danton, 1991
Acute oral, rat	Metam-potassium (UCB) B n° V814/2207KF, 54.7% w/w	Sulaiman, 2004
Acute dermal, rat	Metam-potassium (UCB) B n° V814/2207KF, 54.7% w/w	Sulaiman, 2002
Acute inhalation, rat head and nose	Metam-potassium (UCB) B n° V814/2207KF, 54.7% w/w	Venugopola Rao, 2002
Acute eye irritation, rabbit	Metam-potassium (UCB) B n° V814/2207KF, 54.7% w/w	Prakash, 2002
Skin sensitization- M&K test	Metam-potassium (UCB) batch n° V814/2207KF, 54.2% w/w	Merkel, 2002
90-day in drinking water, mouse at 0.018, 0.088, 0.35, 0.62 mg/mL	Metam-sodium (BASF) B n° Y06930/007 and Y06930/008, 525.54 g/L	Whiles, 1991
90-day in drinking water, rat at 0.018, 0.089 and 0.443 mg/ml	Metam-sodium (BASF) B n° Y06930/007 and Y03930/008; 525.54 g/L	Allen, 1991
90-day, gavage, dog at 1, 5 and 40 mg/kg bw/d	Metam-sodium (BASF) B n° BAS 005 00N; CTL ref n° Y06930/008 43.15%	Brammer, 1992
1-year, gavage, dog at 0.05, 0.1 or 1 mg/kg bw/d	Metam-sodium (BASF) B n° BAS 005 00N; CTL n° Y06930/008; 525.54 g/L (43.148% w/w)	Brammer, 1993
90-day, inhalation, rat at 6.5, 45, 160 mg/m ³	Metam-sodium (Stauffer: Vapam technical) B n° EHC 0355-21; 32.7%	Knapp, 1983

Type of test Test species Used doses	Test substance (source) Batch number Purity	References
21-day, percutaneous, rabbit at 31.25, 62.5 or 125 mg/kg bw/d	Metam-sodium (BASF) B n° BAS 00500 N; 510 g/L (42.2%)	Leuschner, 1979
<i>Salmonella typhimurium</i> strains TA 92, TA 98, TA 100, TA 1535, TA 1537 and TA 1538	Metam-sodium (BASF) B n° BAS 005 00N ; ZH130585; 42.2% (1.0 % impurity 1)	Engelhardt, 1987
CHO cells HGPRT locus	Metam-sodium (BASF) Batch n° ZH130585; 42.2% (56.8 % H ₂ O ; 1.0 % impurity 1)	Engelhardt, 1987
CA in human lymphocytes	Metam-sodium (BASF) Batch n° ZH130585; 42.2% (56.8 % H ₂ O ; 1.0 % impurity 1)	Gelbke and Engelhardt, 1987
CA in human lymphocytes	Metam-sodium (Zeneca WRC) B n° WCR 14989-13-01 ; 32.3%	Mackay, 1996
<i>In-vitro</i> UDS	Metam-sodium (BASF) B n° BAS 005 00N ; ZH130585; 42.2% (1.0 % impurity 1)	Cifone, 1987
<i>In-vivo</i> Micronucleus test Bone marrow cells CD1-mice	Metam-sodium (Zeneca WRC) B n° WCR 14989-13-01; 32.3%	Barber and Mackay, 1996
Cytogenetic study <i>in-vivo</i> Bone marrow cells of ♂ and ♀ Chinese hamsters	Metam-sodium (BASF) B n° ZH130585; 42.2% (1.0 % impurity 1)	Gelbke and Engelhardt, 1987
104 weeks, drinking water, rat, at 0.019, 0.056 and 0.19 mg/mL	Metam-sodium (BASF) B n° BAS 005 00N 90-2; 525.54 g/L (43.148% w/w)	Allen, 1991 Rattray, 1994
24 months, drinking water, mouse, at 0.019, 0.074 or 0.23 mg/mL	Metam-sodium (BASF) B n° BAS 005 00N; 525.54 g/L (43.148% w/w)	Horner, 1994 Pistel, 1994
2 generation, drinking water, rat, at 0.01, 0.03 or 0.1 mg/mL	Metam-sodium (BASF) B n° BAS 005 00N; CTL n° Y06930/008 526 g/L (43.148% w/w)	Milburn, 1993
Developmental rat, gavage d6-15, at 0, 10, 40, 120 mg/kg bw/d	Metam sodium (BASF) B n° ZH 130585; 517.3 g/L (42.2%) (1.0 % impurity 1)	Hellwig and Hildebrand, 1987
Developmental rat, gavage d7-16, at 0, 5, 20 or 60 mg/kg bw/d	Metam sodium (BASF) B n° BAS 005 00N; CTL Y06930/008; 43.15% w/w	Tinston, 1993
Developmental rabbit, gavage d8-20, at 0, 5, 20 or 60 mg/kg bw/d	Metam sodium (BASF) B n° BAS 005 00N; CTL n° Y06930/008; 43.15% w/w	Hodge, 1993
Developmental rabbit, gavage d6-18, 0, 10, 30 or 100 mg/kg bw/d	Metam sodium (BASF) B n° 85/232; ZH130585; 42.2% w/w (1.0 % impurity 1)	Hellwig, 1987
Acute neurotoxicity rat	Metam-sodium (US Task Force) B n° not reported 43.15 %	Lamb, 1993
Subchronic neurotoxicity rat	Metam-sodium (BASF) B n° not reported 525.54 g/L (43.15 %)	Allen, 1994

Clarification:

Notifier explicitly states that the method of manufacture of these specific (eco)tox batches were not expected to be different. The method of manufacture of the Metam Task Force source (including Taminco, Amvac, Buckman, TKI and formerly Zeneca/Stauffer and BASF) did not change significantly for the last 30 years. In addition, as the tested a.s. was in many cases not of a higher purity (42-44%) than the source for which authorisation is sought (maximum 44%), it is plausible that a worst-case condition was tested. Further explanation about the impurities (especially for the issue of genotoxicity hazard assessment) is provided in the confidential section (Vol. 4, annex C).

For the RMS, neither of the existing impurities of the 5B would pose an undue genotoxic hazard. Also the slightly lower concentration of Metam in the former Stauffer source (32% vs. 42%), was not considered critical in the hazard evaluation of the technical a.s. Metam-sodium.

There was little information concerning the level in most tox batches of the 3 significant (>0.1%) impurities, recovered in the 5B.

However, the level of the potential relevant impurity 1 was known in one batch (ZH130585) tested in various studies (see yellow highlight in table). In this batch, the level was demonstrated to be 1% (10 g/kg, or 12 g/L). In the specification of the notifier, impurity 1 was set at 0.3% (2.9 g/kg or 3.6 g/L) maximum. This implies that this impurity was covered for these studies.

Further information was transmitted on the level of impurity 1 in a former BASF batch of 1983, where a 600 g/L concentrate was reported to contain 0.21% of impurity 1, thus about 0.18% in 510-525 g/L batches used around that period. Although the level was marginally slighter than the specification, impurity 1 could be considered covered toxicologically to a certain extent.

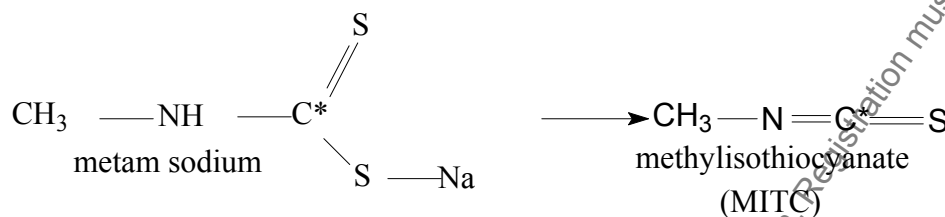
In general, RMS considered it plausible that the toxicological batches were representative for impurity 1, and thus further data requirements are not necessary.

The other impurities 2 and 3a were not considered to be of a higher toxicity than the a.s. itself (see confidential information from original DAR).

B.6.1 Absorption, distribution, excretion and metabolism (toxicokinetics) (Annex IIA 5.1)

Metam-sodium, which is an N-methyl dithiocarbamate, is stable in its dry, crystalline state, and in concentrated aqueous solution. When in dilute aqueous solution or in contact with moist soil, metam sodium rapidly decomposes to methyl isothiocyanate (MITC) which accounts for its fumigant activity. The same is true for metam potassium.

- The proposed specifications cannot be compared to the purity and impurity profile of the material used in tox and ecotox studies, since as yet, no information on the latter is provided.



* Position of ^{14}C .

Findings:**Oral absorption and excretion of metam:**

Results reported in table B.6.1-1 show that 84-86% of radioactivity is excreted within the first 24 h after ingestion, urine and expired air representing the major routes of excretion and fecal excretion representing a minor route. Table B.6.1-2 represents mean radioactive concentration in plasma as a function of time and shows that highest plasmatic concentration is obtained at approximately 1 hour post dosing. Half live of elimination ranged from 60.8 to 74.1 hours.

These results suggest that oral absorption is important and rapid representing a mean of 84% of the dose. Although absorption was just as extensive after the high dose as after the low dose, when the dose is increased by, a factor of 10, urinary excretion is slightly reduced with a concomitant increase of excretion via expired air. This could result from saturation of one of the metabolic pathways as suggested by the increased excretion of methylisothiocyanate (MITC) observed after high oral dose administration.

There is no apparent sex difference in the excretion pattern of metam.

When the dose increased from 10 to 100 mg/kg bw, production of CO_2 was decreased, and formation of MITC was increased from 0.37% to 24%. Production of CS_2 or COS was independent of the administered dose.

The results indicate that oral absorption within 24 h is rapid and important and metabolism is high (Table B.6.1-1a).

Table B.6.1-1: mean excretion of radioactivity after oral administration of a low or a high oral dose metam in rats as a function of time.

Endpoint/time	Cumulative excretion as % of dose			
	10 mg/kg bw		100 mg/kg bw	
	M	F	M	F
Urine				
8	24.19	16.04	17.83	19.17
24	46.49	53.34	33.83	38.34
48	49.46	56	36.01	41.07
72	50.58	56.88	36.65	41.69
96	51.1	57.33	36.9	41.95
120	51.49	57.64	37.08	42.12
144	51.8	57.88	37.22	42.26
168	52.02	58.09	37.34	42.42

Endpoint/time	Cumulative excretion as % of dose			
	10 mg/kg bw		100 mg/kg bw	
	M	F	M	F
Feces				
24	2.98	0.83	0.96	0.66
48	4.01	2.42	1.63	1.26
72	4.26	2.64	1.76	1.44
96	4.34	2.76	1.8	1.48
120	4.39	2.82	1.84	1.51
144	4.43	2.86	1.86	1.53
168	4.46	2.89	1.88	1.55
Expired air: trap1(methyl isothiocyanate)/ trap 2 (CO₂)/ trap 3 (CS₂ or COS)*				
24	0.37/18.44/17.99	1.12/17.03/13.55	23.91/6.68/20.41	23.39/5/17
Sum of 3 traps	36.8	31.7	51	45.39
48	0.40/19.35/18.34	1.22/17.91/13.8	24.48/7.08/21.23	23.96/5.43/17.57
Sum of 3 traps	38.09	32.93	52.79	46.96
72	0.43/19.59/18.35	1.25/18.14/14	24.54/7.19/21.34	24.04/5.54/17.63
Sum of 3 traps	38.37	33.39	53.07	47.21
Total absorbed dose (%)				
24 h	83.3	85.04	84.83	83.73
Tissues	2.01	1.75	1.17	1.32
Cage washings	0.10	0.05	0.06	0.04
Total recovery : % of dose	96.96	95.95	93.50	92.55

*Trap 1 was intended for the trapping of methylisothiocyanate in expired air. Trap 2 was prepared for absorption of CO₂ and trap 3 for COS or CS₂.

Table B.6.1-2: mean concentration of radioactivity in plasma of rats after a single oral dose of metam-sodium at 10 and 100 mg/kg bw.

Time (hours)/dose	µg/ml			
	10 mg/kg bw		100 mg/kg bw	
	M	F	M	F
Pre-dose	<0.019	<0.019	<0.13	<0.13
0.25	1.08	1.19	10.6	11.2
0.5	1.57	1.68	10.4	8.09
1	1.57	1.84	11	11.2
2	1.19	1.61	11.0	9.87
3	0.979	1.33	7.23	9.48
4	0.845	1.06	6.29	9.22
6	0.776	0.884	6.04	8.7
24	0.334	0.440	2.96	5.42
48	0.197	0.283	1.49	2.65
72	0.139	0.226	0.93	1.59
96	0.102	0.166	0.66	1.18
120	0.074	0.126	0.51	0.83
168	0.043	0.081	0.28	0.49
240	0.022	0.049	0.15	0.26
Mean ± SD	60.8	74.1	61.7	64.2

Oral absorption and excretion of MITC:

ADME study with MITC was provided as Metam sodium rapidly decomposes to methyl isothiocyanate (MITC).

After oral administration of a low dose of MITC, a mean of 97% of the dose was excreted within 24 h. After high dose exposure, excretion was slightly reduced to 88-89% within 24 h. Reduced excretion was related to the level of expired ¹⁴C- CO₂ which was strongly decreased.

Urinary excretion (80-82% of the dose) represented the main route of elimination, followed by expired air (7.5-16%). Fecal excretion is a minor pathway (1.5-2.3%). There was no apparent effect of sex on the excretion rates (table B.6.1-3).

C_{max} was slightly higher in females after low oral dose than in males and was obtained after 0.5 hr after low or high dose for males and females (table B.6.1-4).

These data support a high and rapid oral absorption of MITC reaching more than 90% of the administered dose within 24 h.

Table B.6.1-3: mean excretion of radioactivity after oral administration of a low or a high oral dose MITC in rats as a function of time.

Endpoint/time	Cumulative excretion as % of dose			
	4.4 mg/kg bw		33 mg/kg bw	
	M	F	M	F
Urine				
8	71.43	73.65	58.76	54.62
24	80.68	82.45	81.69	80.13
48	82.51	84.29	84.47	83.46
72	83.46	85.15	85.58	84.5
96	83.85	85.52	86.01	84.92
120	84.11	85.81	86.34	85.19
144	84.27	86.13	86.77	85.39
168	84.42	86.35	87.08	85.58
Feces				
24	1.99	0.66	1.13	0.93
48	2.33	1.01	1.60	1.47
72	2.50	1.1	1.73	1.65
96	2.59	1.21	1.79	1.71
120	2.65	1.26	1.84	1.75
144	2.69	1.31	1.91	1.79
168	2.73	1.36	1.95	1.83
Expired air: trap1(methyl isothiocyanate)/ trap 2 (CO₂)/ trap 3 (CS₂ or COS)*				
24	0.69/15.24/0.04	1.24/14.09/0.04	0.49/6.78/0.29	1.20/6.53/0.33
Sum of 3 traps	15.97	15.37	7.56	8.06
48	0.85/15.87/0.06	1.42/14.69/0.06	0.63/7.18/0.37	1.50/7.04/0.43
Sum of 3 traps	16.78	16.17	8.18	8.97
72	0.95/16.08/0.07	1.5/14.87/0.08	0.72/7.32/0.42	1.66/7.23/0.47
Sum of 3 traps	17.1	16.45	8.46	9.36
Total absorbed dose (%)				
24 h	96.65	97.82	89.25	88.19
Tissues	2.2	1.86	1.71	2.29
Cage washings	0.15	0.07	0.18	0.15
Total recovery : % of dose	106.59	106.16	99.37	99.22

*Trap 1 was intended for the trapping of methylisothiocyanate in expired air. Trap 2 was prepared for absorption of CO₂ and trap 3 for COS or CS₂.

Table B.6.1-4: mean concentration of radioactivity in plasma of rats after a single oral dose of MITC at 4.4 and 33 mg/kg bw.

Time (hours)/dose	µg/ml
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	4.4 mg/kg bw		33 mg/kg bw	
	M	F	M	F
Pre-dose	<0.009	<0.009	<0.08	<0.08
0.25	1.33	1.56	8.47	8.95
0.5	1.53	1.60	10.6	11.4
1	1.14	1.45	9.67	11.4
2	0.599	0.748	6.65	7.93
3	0.428	0.478	4.38	7.26
4	0.313	0.388	3.69	5.0
6	0.233	0.288	2.44	3.28
24	0.143	0.185	1.01	1.18
48	0.094	0.153	0.57	0.73
72	0.063	0.091	0.39	0.59
96	0.047	0.076	0.32	0.38
120	0.039	0.059	0.24	0.28
168	0.022	0.049	0.15	0.18
240	0.012	0.022	0.08	0.09

Distribution of metam:

Residual tissue radioactivity was measured at sacrifice. The results reported in table B.6.1-5 show that low levels of radioactivity remain in all the organs, and that the highest level of radioactivity was in thyroid followed by the organs involved in metabolism and excretion, such as liver, kidney and lungs. These results suggest a high affinity for thyroid.

Table B.6.1-5: tissues and organ distribution of metam-sodium at sacrifice time (168 h).

Endpoint/dose	µg equivalents/g tissue			
	10 mg/kg bw		100 mg/kg bw	
	M	F	M	F
Testes/ovaries	0.036	0.340	0.25	2.12
Plasma	0.044	0.072	0.23	0.33
GI tissues	0.060	0.098	0.30	0.42
Spleen	0.067	0.125	0.62	0.93
Brain	0.071	0.086	0.46	0.61
Fat	0.076	0.048	0.32	0.27
Eyes	0.077	0.084	0.48	0.51
Pancreas	0.077	0.116	0.53	0.66
Muscle	0.082	0.089	0.45	0.54
Marrow	0.090	0.156	0.42	0.71
Carcass	0.146	0.172	1.05	1.53
Heart	0.168	0.247	0.95	1.23
Whole blood	0.219	0.263	2.04	3.01
Adrenal gland	0.210	0.225	1.58	1.78
Lungs	0.323	0.924	1.50	3.46
Kidney	0.734	1.29	3.49	6.59
Liver	0.765	0.245	3.58	1.20
uterus	-	0.118	-	0.59
Thyroid gland	1.28	3.09	6.24	7.55

Distribution of MITC:

Overall, at 168 h, residual radioactivity remained in all organs at quite similar levels except for excretory organs such as liver, kidneys and lungs where slightly higher levels of radioactivity remained. As like as metam, MITC was detected at the highest level of radioactivity in thyroid (table B.6.1-6).

Table B.6.1-6: tissues and organ distribution of MITC at sacrifice time (168 h).

Endpoint/dose	$\mu\text{g equivalents/g tissue}$			
	4.4 mg/kg bw		33 mg/kg bw	
	M	F	M	F
Testes/ovaries	0.01	0.041	0.08	0.50
Plasma	0.013	0.028	0.09	0.14
GI tissues	0.036	0.068	0.25	0.41
Spleen	0.023	0.036	0.20	0.28
Brain	0.024	0.035	0.21	0.29
Fat	0.012	0.011	0.16	0.12
Eyes	0.034	0.027	0.29	0.41
Pancreas	0.031	0.040	0.22	0.29
Muscle	0.021	0.026	0.15	0.20
Marrow	0.024	<0.078	<0.24	0.62
Carcass	0.079	0.080	0.55	0.86
Heart	0.038	0.059	0.30	0.51
Whole blood	0.062	0.094	0.67	0.91
Adrenal gland	0.058	0.060	0.38	0.81
Lungs	0.037	0.077	0.41	1.04
Kidney	0.080	0.137	0.76	1.57
Liver	0.119	0.107	0.89	0.65
uterus	-	0.024	-	0.20
Thyroid gland	0.248	0.370	1.58	4.07

Metabolism of metam:

Patterns of metabolites in urine were similar at the low and high dose levels. Enzyme treatment had no significant effect on the urine metabolites. At low or high dose level, total urinary radioactivity was resolved into five major regions as detected by TLC (table B.6.1-7).

TLC resolved methanol extractable radioactivity from male and female livers into 4 major components. Components M1 and M2 had the same chromatographic properties as M1 and M2 found in rat urine while M6 was not detected in rat urine. TLC resolved kidneys extractable radioactivity into three major components: the principal was M2. M6 was not detected in the kidney extract.

Metabolites M2, M4 and M5 resulted from MITC conjugation with GSH.

Major metabolite M5 was N-acetyl-S-(N-methylthiocarbamoyl)-L-cysteine, which represented 16-25% of the dose. The second metabolite, accounting for about 5-10% of the administered dose, was not exactly characterized but there is evidence that it is a similar type of conjugate as the major metabolite.

Metam is extensively converted to methyl isothiocyanate *in vivo*, which is further conjugated with GSH. The other significant metabolic pathway is formation of CS₂ as may be expected for a thiocarbamate. Release of CS₂ could be related to the acidic conditions existent in the stomach of the rat (pH 3.8-5) following oral ingestion.

Fig 6.1 shows the postulated biotransformation of metam.

Table B.6.1-7: proportion of radioactive components in 0-24 h urine of rats after oral low and high dose administration of metam.

Approx Rf of components	% of the administered dose							
	10 mg/kg bw				100mg/kg bw			
	Untreated		Enzyme treated		Untreated		Enzyme treated	
Dose	M	F	M	F	M	F	M	F
Metabolites								
Urine 0-24 h								
0.04 M1	6.6	7	6.3	5.2	3.2	2.5	2.5	2.3
0.13 M2	5.5	4.9	5.2	5	3.3	2.8	2.9	2.7
0.24 M3	3.2	2.4	4.2	3	1.3	1.1	1.2	1.1
0.42 M4	8.3	5.1	7.7	8.9	5.9	6.1	5.7	5.5
0.50 M5	16.1	27.2	16	25.1	17.7	23.3	18.8	21.1

others	6.9	6.7	7.2	6.2	2.6	2.4	2.6	2.7
Liver at 0.5h	% of the tissue radioactivity							
0.04 M1					31.6	23.2		
0.13 M2					7.7	14.7		
0.50 M5					11.9	10.8		
0.88 M6					2.0	12.1		
Others					12.7	15.3		
Kidneys at 0.5 h								
0.04 M1					13.1	6.4		
0.13 M2					45	43.1		
0.50 M5					7.7	4.8		
0.88 M6					ND	ND		
Others					16.2	12.7		

Metabolism of MITC:

Data reported in table B.6.1-3 suggest that a minor part of MITC is excreted unchanged via expired air (0.5-1.25%), 6.5-16% is excreted as CO₂, COS and CS₂ being minor metabolites (0.04-0.5% of the dose).

Patterns of urinary metabolites were similar at the low and high dose levels and were similar to those found after oral doses of metam sodium. Metabolite M5 and M4 were the most abundant in male and female rats. The pattern of metabolites was unchanged by enzyme incubation except for M3, which was slightly increased after low dose treatment. A much higher formation of M5 was seen in urine of rats after MITC administration when compared to metam sodium, and is related to conjugation with GSH.

TLC resolved methanol extractable liver radioactivity into 4 major components. Metabolite M5 accounted for 13.3-18%. Metabolites M1 and M2 had the same chromatographic properties as M1 and M2 found in urine and M6 was not detected in rat urine. Kidney extractable radioactivity resolved into 3 major components with M1 being the most important. The major urinary compound M5 was not detected in kidneys.

Comment from RMS: According to the open literature, a principal detoxification step for metam and MITC is their conversion to the GSH conjugate. MITC reacts readily with GSH without GS transferase, whereas the metam reaction is catalyzed by GS transferase without the requirement of MITC as an intermediate. Each compound results ultimately in a large amount of the mercapturate in urine with methylamine as an additional product for metham but less so for MITC. Metabolism of methylamine could give rise to formaldehyde and according to the authors, ¹⁴C residues in tissue could derive from extensive degradation to formaldehyde and formic acid (Lam et al., 1993). See table B.6.1-9.

Table B.6.1-8: proportion of radioactive components in 0-24 h urine of rats after oral low and high dose administration of MITC.

Approx Rf of components	% of the administered dose							
Dose	4.4 mg/kg bw				33mg/kg bw			
Metabolites in:	Untreated		Enzyme treated		Untreated		Enzyme treated	
Urine 0-24 h	M	F	M	F	M	F	M	F
0.04 M1	2.1	2.1	1.9	1.8	1	1.1	1.3	1.2
0.13 M2	4.1	4.1	4.6	4.8	4.2	4.1	10.2	10.9
0.24 M3	2.6	1.7	4.7	2.9	2	2.5	2.1	2.3
0.42 M4	9.3	6.7	7.6	6.4	4.8	5.9	5.7	5.9
0.50 M5	55.5	62.2	56.9	60.7	66.2	63.8	57.4	56.9
others	7	5.7	5.2	5.8	3.6	4.2	3.6	3
Liver at 0.5h	% of the tissue radioactivity							
0.04 M1					32.5	31.6		
0.13 M2					8.8	16.1		
0.24 M3					ND	ND		
0.50 M5					18.3	13.3		
0.88 M6					2.2	1.0		
Others					7.2	5.0		
Kidneys at 0.5 h								
0.04 M1					67	57.1		
0.13 M2					6.4	21.2		
0.24 M3					1.7	2.1		
0.50 M5					ND	ND		
0.88 M6					ND	ND		
Others					11.9	7.6		

Additional information from open literature:**- Metabolism in rats and mice of the soil fumigants metham and methyl isothiocyanate (Lam et al, 1993).****Findings:**

Excretion of radioactivity after ip administration of metam, MITC or methylamine, radioactivity was mainly in urine. Fecal excretion was low. A small part was exhaled as $^{14}\text{CO}_2$. The recovery was insufficient in the mice treated with Metam and Methylamine, which was explained by the authors by the possibility that not all expired radioactivity was trapped. About 85-90% of the dose of the carcass radioactivity was not extractable with MeOH, suggesting a covalent binding to the biological macromolecules of the matrix. Radioactivity was widely distributed among the tissues, with a preference for liver, lung and kidney. At 48h, the average level of tissue radioactivity was 10-30% of the dose.

Table B.6.1-9: ^{14}C -Radioactivity 48h after ip administration of Metam, MITC, or Methylamine to male mice.

Compartment	Recovery of radioactivity % of administered dose		
	Metam	MITC	Methylamine
Urine	58	80.2	35.4
Faeces	5.9	4.8	1.6
$^{14}\text{CO}_2$	4.1	3.8	5.0
Carcass	7.5	6	21
tissues	20	10	29
Total	75.5	94.7	63.0

A principal detoxification step for metam and MITC is their conversion to the GSH conjugate. This reaction occurs without GST for MITC, while metam requires the enzyme. This reaction results ultimately in a large amount of mercapturate in urine with methylamine as additional product. The vast majority of the unknown

radioactivity, particularly in mice, consisted of polar metabolites. There was no evidence of oxidative conversion to isocyanides, as such mercapturate conjugate ($\text{CH}_3\text{NHC}(\text{O})\text{S-R}$) was not found in the urine (data not shown).

The results presented in table B.6.1-10 show that, although rat and mice have several metabolites in common, metabolism differs quantitatively and qualitatively. There are large amounts of unidentified metabolites in mice, which are polar.

Table B.6.1-10: metabolites in the 0-48 h urine after i.p. administration of ^{14}C -Metam, ^{14}C -MITC, and ^{14}C -Methylamine to rats and mice.

	% of the urinary radioactivity		
Rat	Metam (167 mg/kg bw)	MITC(33 mg/kg bw)	Methylamine (1.5 mg/kg bw)
Mercapturate conjugate	34	26	
Methylamine	5	2	13
polar metabolites	26	61	
unidentified	35	11	87
Mouse	Metam (7 mg/kg bw)	MITC(4 mg/kg bw)	Methylamine (1.7 mg/kg bw)
Mercapturate conjugate	3	2	
Methylamine	14	2	25
polar metabolites	77	85	
unidentified	6	11	75

Materials and methods:

♂ Swiss-Webster mice received ^{14}C -Metam (7.0 mg/kg b.w.), ^{14}C -MITC (4.0 mg/kg b.w.), or ^{14}C -Methylamine (1.7 mg/kg b.w.) by i.p. injection.

Excretion of radioactivity was measured in urine, faeces and via expired air ($^{14}\text{CO}_2$). Residual radioactivity was measured in carcass. Tissue distribution was investigated.

Urine aliquots were retained for the determination of metabolites (either direct or after derivatisation with benzoylchloride) by HPLC (UV, 220 nm) coupled with radiodetection.

♂ Sprague Dawley rats received ^{13}C -Metam (167 mg/kg b.w.) or ^{13}C -MITC (33 mg/kg b.w.) either by (a) gavage, by (b) i.p. injection or by (c) i.p. injection after bile-cannulation. Radiolabel in urine (6-8h) and bile (8h) was characterized by ^{13}C -NMR. The methylamine and mercapturate metabolites, obtained in the urine of rats dosed with ^{13}C -Metam, were identified with standards.

Metam was dissolved in water (all experiments), MITC in DMSO (mouse experiments) or corn oil (rat experiments). The radiolabel –either ^{14}C (*) or ^{13}C (*) – positions in Metam, MITC, Dazomet and Methylamine were as depicted in the following figure:

$\text{H}_3^*\text{C}-\text{NH}-\overset{\text{S}}{\underset{\text{ }}{\text{C}}}-$	$\text{H}_3^*\text{C}-\text{N}=\text{C}=\text{S}$	$\text{H}_3^*\text{C}-\text{N}(\text{S}=\text{S})-\text{N}^*\text{CH}_3$	$\text{H}_3^*\text{C}-\text{NH}_2$
^{14}C -Metam	^{14}C -MITC	^{14}C -Dazomet	^{14}C -Methylamine

Overall conclusions:

Oral absorption of metam and its main metabolite MITC is rapid and important reaching a mean value of 85% and 90% of metam or MITC, respectively. Comparing the results from the high and low dose levels, it is clear that metabolism of metam sodium may be saturated at high dose levels resulting in a reduced breakdown of MITC, which is then excreted unchanged via exhaled air.

Tissue distribution is important and suggests that thyroid could be a target organ. Approximately 2.3% of the dose was still detected at 168 h in tissues. Half-life of elimination from plasma ranged from 60.8 to 74.1 hours.

Metabolism is extensive and rapid, suggesting a decomposition of metam into MITC, CO_2 , and COS. MITC is further conjugated to GSH and excreted in urine while CO_2 and COS are excreted via expired air. The other significant pathway for metam is the release of CS_2 , which could be related to the acidic conditions existent in the stomach of the rat (pH 3.8-5) following oral ingestion.

From published studies in the open literature, it appears that, although similar metabolites were identified in rats and mice, non identified part of polar metabolites are more important in mice than in rats suggesting that metabolism of metam and MITC after ip administration, is quantitatively and qualitatively different.

The majority of the material given was excreted within 24-48 h after administration with minor portions excreted up to 168 h after dosing.

- The biokinetics and metabolism of ^{14}C -metam sodium in the rat (Hawkins, 1987).

Guidelines: not fully in agreement with Dir 87/302/EEC Annex V B or OCDE test guideline 417(1984).

Deviation from official protocol: the repeat dose study was realized with dazomet; toxicity was not reported and oral absorption was not estimated.

GLP: yes (no attest of competent authority)

Material and Methods: 22 COBS (SD) CD rats/sex received by gavage a single dose of ^{14}C -metam sodium (batch n° 216/5/1; specific activity 64.5 $\mu\text{Ci}/\text{mg}$ with radiochemical purity >99%) was diluted with metam sodium non radiolabelled (batch n° CH 686001) to a suitable specific activity (15520 dpm/ μg) for the 10 mg/kg dose and (2227 dpm/ μg) for the 100 mg/kg dose. Doses were prepared in aqueous solution. 10 Animals were housed in metabolism cages and urine was collected in solid CO_2 -cooled containers. Feces were collected. Expired air was collected during 24 h intervals by passing the air through a series of 3 traps containing ethoxyethanol, 20% w/v aqueous NaOH and Viles reagent (ethanol: diethylamine: triethanolamine 1000:1:20 solution containing cupric acetate). Blood was taken before sacrifice at day 7.

10 animals were used for the excretion retention study and 10 animals were used for plasma concentration of radioactivity.

Urine samples as well as liver and kidney samples were prepared for measurement of radioactive components by thin-layer chromatography. Urine and bile samples were submitted to enzymatic treatment (β glucuronidase and aryl sulfatase). Liver and kidney samples were extracted 3 times with methanol.

TLC of urine and tissue samples was carried out on pre-layered plates Kieselgel with developing solvent CHCl_3 /methanol/water/formic acid (75:25:3:3).

The same experiment was performed using MITC: ^{14}C MITC (specific activity: 16.33 $\mu\text{Ci}/\text{mg}$) which was 95% radiochemical pure and chemically pure MITC (Aldrich) were given orally to 22 COBS (SD) CD rats/sex. MITC was dissolved in Na carboxymethyl cellulose in order to obtain 4.4 and 33 mg/kg bw dose levels.

The study is accepted.

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Table B.6.1.1: Nomenclature of identified or tentatively identified metabolites of Metam in the rat

Code	Chemical name
Metam-sodium	sodium-N-Methyldithiocarbamate
MITC	methylisothiocyanate
COS	carbonyl sulphide
CS_2	carbon disulphide
CO_2	carbon dioxide
M2	S-(N-methylthiocarbamoyl)-L-cysteine
M4	S-(N-methylthiocarbamoyl)-pyruvic acid
M5	N-acetyl-S-(N-methylthiocarbamoyl)-L-cysteine

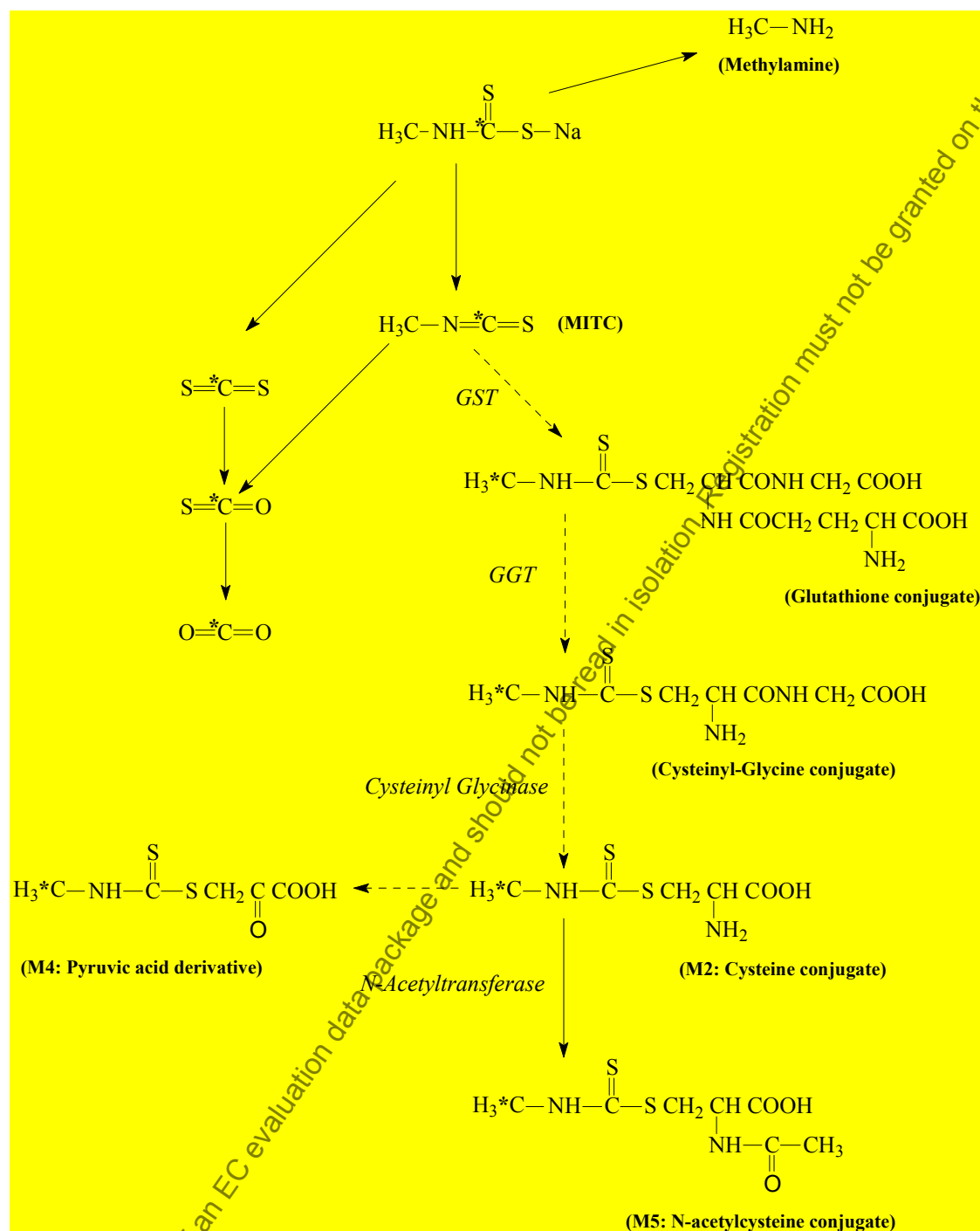
Fig (1): Postulated metabolic pathway for ^{14}C -metam sodium and methyl isothiocyanate in the rat.

In short, exhaled metabolites were MITC, CS_2 , COS and CO_2 , while mercapturate, glutathione, cysteine, and acetylcysteine conjugates of MITC were recovered in the urine.

Figure B.6.1.1: Proposed metabolic pathway of ^{14}C -Metam in the rat

Captions:

- GST: Glutathione S-Transferase
- GGT: Gamma-glutamyltranspeptidase)
- Dashed arrows: precedes probable intermediate; CS_2 , COS and CO_2 recovered in the expired air



Captions:

GST: Glutathione S-Transferase;

GGT: gamma-Glutamyltranspeptidase;

dashed arrows: precedes probable intermediate; CS₂, COS and CO₂ recovered in the expired air.

B.6.2 Acute toxicity including irritancy and skin sensitization (Annex IIA 5.2)

Batch analysis was provided for batches 32E0410, 32E2806, 32E1906, 32E0208 and 32E1307 with a proposed metam content of 508-514 g/L.

Metam sodium is synthesized in aqueous solution, and then diluted with water, if necessary. There is no technical metam sodium as such since the technical material is never isolated.

Metam potassium is an alternative to metam sodium. As with metam sodium, the gaseous active metabolite, MITC (methyl isothiocyanate) is formed upon contact with the soil. In some regions, however the use of the potassium counter ion is preferred to sodium.

B.6.2.1.a Acute oral toxicity of metam sodium (Annex IIA 5.2.1)

- **Acute oral toxicity to rats of metam sodium at 640, 800, 1000 or 1600 mg/kg bw (Baldrick and Healing, 1991a) (study n° 901046D/UCB 366/AC)**

Findings:

A preliminary test was carried out by dosing 2 rats/sex at 1000 mg/kg bw and the acute median lethal dose of metam sodium was in the region of 1000 mg/kg bw for males and greater than 1000 mg/kg bw for female rats.

Main study:

Mortality:

Dose (mg/kg bw)	Death		Day or hours after dosing	
	M	F	M	F
640	0	0		
800	1	0	Day 2	
1000	5	5	4 day 4, 1 after 5 h	4 day 2, 1 after 5 h
1600	5	5	4 after 4 h; 1 after 1 h	3 after 4 h; 2 after 5 h

Body weight: was not affected in surviving male animals; surviving females of the group 800 mg/kg bw had decreased body weight.

Clinical observations: all animals had piloerection, hunched posture, abnormal gait, lethargy, decreased respiratory rate, ptosis, pallor of the extremities, increased salivation. Clonic convulsions and prostration affected animals at 1000 and 1600 mg/kg bw.

Necropsy: no macroscopic abnormalities were seen.

Conclusions:

The acute medium lethal oral doses with 95% confidence limits were estimated to be:

Male and female rats combined 896 mg/kg bw.

Males only: 870 mg/kg bw

Females only: 924 mg/kg bw

Metam sodium should be classified as harmful and assigned the symbol **Xn**, risk phrase **R22**.

GLP: yes (no attest of competent authority)

Guidelines: in agreement with Dir 92/69/EEC.

Material and Methods: 5 CD rats/sex received metam sodium (510 g/L) at 640, 800, 1000 or 1200 mg/kg by gavage.

Statistical analysis: the data obtained did not permit the fitting of a probit line using the standard method. However, it was possible to fit a line if a fixed slope was assumed and a value of 8.333 was used. A test for difference between the sexes was carried out.

The study is accepted.

- **The acute oral toxicity of metam fluid in rats (Leuschner, 1977)**

Findings and conclusion:

At 2.15 ml, all rats died. At 1.78 ml, 6 males and 8 females died; at 1.47ml, 3 males and 4 females died and at 1.21ml, 1 male died.

No signs of toxicity were seen at 0.82 ml. Animals showed sedation (1.000 ml/kg), reduced food intake, abdominal position, comatose conditions (1.210 ml/kg), ataxia, ptosis, dyspnoea (1.470 ml/kg). Dead occurred

in comatose conditions after tonic-clonic convulsions within 4-24 hr. Surviving animals recovered within 2-4 days. No specific pathological findings were reported.

LD50 male rats = 1550 ml/kg

LD50 females = 1580 ml/kg

GLP: no

Guidelines: not fully in agreement with Dir 92/69/EEC.

Deviation from official protocol: no raw data were provided, no detailed reports of experimental results. The purity of metam is not reported.

Material and Methods: 10 Sprague-Dawley rats/sex received by gavage metam-fluid (??) dispersed in 1% aqueous methylhydroxyethylcellulose at 0.825, 1.000, 1.210 ml, 1.47, 1.78, 2.15 ml/kg by gavage.

Statistical analysis: LD50 was calculated according to Litchfield and Wicoxon.

The study is accepted as additional information.

Form the open literature:

- ***In vivo and in vitro* hepatotoxicity and GSH interactions of N-methyldithiocarbamate in the rat (Thompson et al, 2002)**

Findings: after a single oral dose of metam at 2 mmol/kg, blood ALT and AST were increased while γ GT and bilirubin were not altered. Infiltration of mononuclear inflammatory cells into the region surrounding the central hepatic vein occurred in all 3 rats.

In an *in vitro* experiment using liver hepatocytes incubated with 2 mM metam for 3 hr, a concentration dependent decrease in intracellular GSH and an increase in extracellular GSH were reported. Intracellular GSSG was not altered. The authors explain this effect by a conjugation of MITC with GSH and a subsequent transport out of the cell.

Dose/effect	2 mmol/kg
ALT	↑785%
AST	↑1973%
Liver histopathology:	
Mononuclear cell infiltration	3/3
Necrosis	3/3

Conclusion: metam is a liver toxicant in rats. Metam is metabolized into MITC, which is further conjugated with GSH and subsequently transported outside the liver cell.

Material and methods: 3 male Sprague Dawley rats were fasted for 18 h prior to being administered a single oral dose of metam (synthesized in the laboratory, high purity) at 2 mmol/kg (358 mg/kg bw) in water. Rats were sacrificed 24 hr later, and blood and liver were taken. Liver was prepared for hepatocyte isolation and culture. An additional experiment was performed with 0.5 (= 89 mg/kg), 1.0 (179 mg/kg), and 1.5 mmol/kg (268.5 mg/kg) metam where blood was taken from rats.

B.6.2.2a Acute percutaneous toxicity of metam sodium (Annex IIA 5.2.2)

- **Acute dermal toxicity to rats of metam sodium at 2 g/kg bw (Baldrick and Healing, 1991b)**

Findings:

One female rat died on day 2 following a single dermal dose of metam sodium of 2 g/kg body weight. A bodyweight loss was recorded for the above decedent and autopsy revealed slight congestion in the subcutaneous region which was green in color. All rats were hypothermic on day 2. A slightly low body weight gain was recorded for 1 male rat.

Terminal autopsy revealed scabs of the subcutaneous region for one male and 3 females, and localized ulceration of the same region in a single male and female.

Sites of application of metam sodium showed well defined to moderate erythema in all males and a majority of surviving females on day 2 only. Slight to moderate edema was present in all surviving rats between days 2 and 3. These dermal reactions were accompanied by staining of the dose site (green yellow) and/or localized hard

white areas of skin in all surviving rats from day 2 of the study. Reactions progressed to scab formation in 2 males and 4 males, which persisted until day 15 in a single male and female.

Conclusions:

Acute dermal LD50 > 2000 mg/kg bw

GLP: yes (no attest of competent authority)

Guidelines: not fully in agreement with Dir 92/69/EEC or 84/449 or OECD 402 (1987-81): the test is a limit test at 2000 mg/kg bw and mortality affected 1/5 females.

Material and Methods: metam sodium (510 g/l) was administered to 5 CD rats/sex at a dose of 2 g/kg bw. The test substance was applied onto the clipped skin and covered with gauze and impermeable dressing for 24 h after which the skin was washed in warm water. The study is accepted.

- **The acute dermal toxicity of metam fluid in rats (Leuschner, 1977)**

Findings and conclusion: according to the author of the study, there were no distinct systemic signs of intolerance reactions. Locally, there were erythema (Draize 1), edema (Draize 4), and exsiccation of the cutis with subsequent necrosis of epidermis, shedding of necrotic layers, and renewed epithelialisation within 15-20 days. At autopsy, only local reactions were seen.

GLP: no

Guidelines: not in agreement with Dir 92/69/EEC, 84/449, or OECD 402 (1987-81).

Deviation from official protocol: no raw data were provided, no detailed reports of experimental results. The purity of metam is not reported. Scores of erythema and edema are not clearly reported.

Material and Methods: metam fluid (??) was applied once for 24 h on the shaved dorsal skin of 10 Sprague-Dawley rats/sex at a dose of 1.59, 2.0 or 2.52 ml/kg bw. The test substance was covered with gauze and impermeable dressing for 24 h after which the skin was washed in warm water.

The study is not accepted.

B.6.2.3a Acute inhalation toxicity of metam sodium (Annex IIA 5.2.3)

- **Acute inhalation toxicity in rats of metam sodium – 4 hours exposure, whole body at 3.03, 1.23, 2.43 and 3.15 mg/L (Jackson and Hardy, 1992)**

Findings:

Mortality: affected 2/5 males at 2.43 mg/L and 4/5 males and 4/5 females at 3.15 mg/L.

Clinical signs: during exposure, signs consistent with exposure to an irritant and toxic aerosol including, partial closing of the eyes, wetness around the eyes, mouth and snout, reddening of the ears, feet and tail, exaggerated respiratory movements and the adoption of a hunched or prone posture. During the observation period, the main signs seen were persistent signs indicative of a long-term effect on the respiratory tract. The signs had disappeared by day 6 at 1.23 mg/L but at higher levels were present throughout the observation period. Also seen was formation of scabs on the scrotal sac and ears, indicative of irritation of the skin.

Body weight was reduced for up to 3 days following exposure.

Food consumption was reduced for up to 9 days following exposure. Water consumption was reduced immediately following exposure, but in females, there was evidence of increased water consumption later.

Organ weight: lung weight/body weight for rats dying was higher than control values.

Lungs were congested and stomach was gas filled.

Microscopy: except for the low dose level, rats showed histopathological lung changes progressing to bronchiolar epithelial necrosis with inflammatory exudates. Some rats treated with 3.15, 3.03 and 2.43 mg/L and all rats treated with 1.23 mg/L surviving to termination showed increased incidence of prominent alveolar macrophages as compared to control.

Conclusions:

LC₅₀ combined = 2.54 mg/L (≅ 686 mg/kg bw)

Males: 2.20 mg/L

Females: 2.95 mg/L

Metam sodium should be classified as harmful by inhalation and assigned the symbol Xn and risk phrase R20.

GLP: yes (no attest of competent authority)

Guidelines: not fully in agreement with Dir 92/69/EEC, 84/449, or OCDE 403 (1981).

Deviation from official protocol: absence of raw data for clinical signs.

Material and Methods: 5 Sprague-Dawley rats/sex/dose were exposed whole body by inhalation to a test atmosphere containing a liquid droplet aerosol generated from metam sodium (510 g/L) at 1.23, 2.43, 3.03 or 3.15 mg/L for a 4-hour period. A control group was exposed to air only.

group	Concentration in air (mg/L)	MMAD	% Respirable (<6µm)
2	3.03	3.1±2.1	76
3	1.23	3.0±2.04	84
4	2.43	3.2±2.05	80
5	3.15	3.2±2.27	76

The study is accepted.

- **Acute inhalation toxicity LC₅₀ 4 hours (rat) liquid aerosol study, head and nose, of metam sodium at 6.8 mg/L (Gelbke and Klimisch, 1986)**

Findings:

Mortality was not seen.

Clinical signs during exposure animals attempt to escape. Reddish discharge from the nose and discharge from eyes were noted. After inhalation, hair in the genital area was covered with reddish crusts as well as part of the nose covered with reddish crusts. Stepping gait was slightly high and respiration was slightly sound.

Body weight gain of male rats was retarded in the first week of observation period but recovered in the second week. Body weight gain of females remained slightly retarded over the total observation period. No organ abnormalities were detected.

Conclusions:

LC50 male and female rat > 6.8 mg/L (≅1836 mg/kg bw). Metam sodium should not be classified by inhalation.

GLP: yes (no attest of competent authority)

Guidelines: in agreement with Dir 92/69/EEC, 84/449, or OCDE 403 (1981).

Deviation from official protocol: absence of raw data for clinical signs.

Material and methods: 10 Wistar rats/sex were exposed to metam sodium (batch n° ZH 130 585, 42.2% aqueous solution) by head-nose to an aerosol air mixture for 4 hours to 6.8 mg/L (MMAD 50% = 2.6 µm; 90.1% was respirable).

The study is accepted.

- **The acute toxicity of metam fluid when administered to rats by inhalation (Leuschner, 1977)**

Findings and conclusion: there were no signs of intolerance reactions and autopsy did not show specific pathological findings. Reduced food intake was observed at top dose. LC50 > 25.6 ppm

GLP: no

Guidelines: not in agreement with Dir 92/69/EEC, 84/449, or OCDE 403 (1981).

Deviation from official protocol: description of the test and of experimental results limited to a summary. Exposure time of 8 hours.

Material and methods: 10 Sprague-Dawley rats/sex were exposed to metam fluid (batch n°?) by head-nose to an aerosol for 8 hours to 16.2, 20.8 or 25.6 ppm.

The study is not accepted.

B.6.2.4a Skin irritation with metam sodium (Annex IIA 5.2.4)

- **Skin irritation to rabbits with metam sodium (Liggett and McRae, 1991a)**

Findings:

<Score erythema> 30min+ 48h + 72 h = 3.2

<Score edema> 30 min + 48 h + 72 h = 1.1

Severe reactions consisting of necrotic lesions accompanied by very slight-to-slight edema were present on day 2. In two animals, the severity of response (necrosis with fissuring and associated hemorrhage) necessitated killing the animals on day 8. In 2 animals, the reactions had ameliorated slightly by day 10 but an inflammatory reaction accompanied by desquamation of *stratum corneum* was still present on day 14. Well-defined erythema accompanied by slight to moderate edema was observed in 2 of the remaining animals on day 1. These reactions slowly resolved and by day 10, the treatment sites were normal.

Conclusions:

Metam sodium is corrosive to the skin. The symbol C and the risk phrase R34 should be assigned.

GLP: yes (no attest of competent authority)

Guidelines: not fully in agreement with Dir 92/69/EEC or 84/449/EEC or OECD 404(1992-81)

Deviation from official protocol: observations limited to the scores; untreated skin was not examined.

Material and Methods: intact skin of 3 New Zealand White rabbits/sex was exposed to metam sodium (510 g/L) at 0.5 ml and skin was semi occluded for 4 h period after which skin was washed with water. The first observation was made 30 minutes after removal of dressing. The study is accepted.

- **Skin irritation to rabbits with metam sodium (BASF, 1988)**

Findings:

<Score erythema> 24h + 48h + 72 h = 3

<Score edema> 24h + 48 h + 72 h = 2.2

Conclusions:

Metam sodium is corrosive to the skin. The symbol C and the risk phrase R34 should be assigned.

GLP: no

Guidelines: in agreement with Dir 92/69/EEC or 84/449/EEC or OECD 404(1992-81)

Deviation from official protocol: observations limited to the scores; untreated skin was not examined.

Material and Methods: intact skin of three White Vienna rabbits (1male and 2 females) was exposed to metam sodium (test n° 82/3; 560 g/L) at 0.5 ml and skin was occluded for 4 h period after which skin was washed with control water. The study is accepted.

B.6.2.5a Eye Irritation with metam sodium (Annex IIA 5.2.5)

- **Eye irritation to rabbits with metam sodium (Giggett and McRae, 1991b)**

Findings:

In unwashed eyes:

<Score cornea >_{24+48+72h} = 0

<Score iris>_{24+48+72h} = 0

<Score conjunctivae redness>_{24+48+72h} = 0.8

<Score conjunctivae chemosis>_{24+48+72h} = 0.2

Conclusions:

Metam sodium is not irritating to eyes.

GLP: yes (no attest of competent authority)

Guidelines: not fully in agreement with Dir 92/69/EEC or 84/449/EEC or OECD 405 (1987-1981).

Deviation from official protocol: observations are limited to scores

Material and Methods: 0.1 ml metam sodium (510 g/L) was placed into the lower reverted eyelid of one eye of 6 New Zealand White rabbits. Eyelids were then held together for 1 sec.

The study is accepted.

B.6.2.6a Skin sensitization with metam sodium (Annex IIA 5.2.6)

- **Delayed contact hypersensitivity in the guinea-pig with metam sodium (Parcell and Denton, 1991)**

Findings:

Metam sodium produced evidence of delayed contact hypersensitivity in 19 guinea pigs.

Conclusions:

Metam sodium is a skin sensitizer. The symbol Xi and risk phrase R43 should be assigned.

Guidelines: not fully in agreement with Dir 96/54/EEC Annex IV C or 92/69-84/449 or OECD 406 (1981-92).

Deviation from official protocol: for challenge, two concentrations were tested per animal. Scores after induction are not reported.

GLP: yes (no attest of competent authority)

Material and Methods: 20 female guinea pigs Dunkin/Hartley strain/ group were exposed to metam sodium (510 g/L).

Preliminary investigation: induction used a 5% v/v in water; topical application was at 2.5% in distilled water and for challenge, topical application used a solution at 1 and 0.5% v/v in water.

Main study:

- Induction phase: metam sodium at 5% in water and metam sodium at 5% in a 50:50 mixture of Freund's complete adjuvant and water. Topical application of 2.5% v/v in water.
- Challenge phase: test and control animals were challenged topically 2 weeks after topical induction using metam sodium at 1% and 0.5% v/v in water applied at two sites of each animal. The challenge sites were evaluated 24, 48, and 72 h after removal of the patches.

The study is accepted.

- Dermal sensitization test with Vapam technical, Buehler test (Mutter, 1987)

Findings and conclusion:

Vapam technical did not induce adverse effects to the guinea pigs, as measured by weight gain, general appearance and behavior. Repeated application of 1% Vapam technical produced erythema, skin flaking and eschar reactions during the induction phase. Challenge application of Vapam technical produced sensitization reactions in guinea pigs induced with this test material. Although the negative control group did not respond to Vapam technical at the first challenge, subsequent applications of Vapam and MITC did elicit sensitization reactions in these guinea pigs. The results support that Vapam is a sensitizer.

Guidelines: not fully in agreement with Dir 96/54/EEC Annex IV C or 92/69-84/449 or OECD 406 (1981-92).

Deviation from official protocol: experimental protocol not clearly reported; limited number animals used.

GLP: no

Material and Methods: 10 guinea pigs Dunkin/Hartley strain/ group were induced over a three-week period and challenged on day 35, 42 and 49.

- Induction phase: Vapam technical at 1% was used for the 10 induction applications of 6 hours for a 3-week period.
- Challenge phase: test and control animals were challenged with Vapam technical at 1% and vehicle. Second challenge with 0.1% vapam technical as well as vehicle and third challenge, animals were treated with Vapam technical, isothiocyanate and vehicle.

The study is accepted as additional information.

B.6.2.1.b Acute oral toxicity with metam potassium (Annex IIA 5.2.1)

- Acute oral toxicity study (acute toxic class method) with metam potassium aqueous solution at 2000 and 200 mg/kg bw in Wistar rats (Sulaiman, 2004)

Findings:

The starting dose was 200 mg/kg bw which is considered as the dose where the mortalities are most likely be produced. The treatment was initiated with females and then with males at the dose of 200 mg/kg bw. Lethargy was observed on day 1 and persisted in 1 male on day 2. There were no pre-terminal deaths and no gross necropsy changes observed the dose of 2000 mg/kg bw was considered and administered to female rats.

Lethargy was observed in G2 group in 1 rat, lethargy and convulsions in another rat and another rat was lethargic and recumbent. All 3 females died.

Body weight: of all surviving rats increased through the observation period.

Necropsy: one female of the group G2 had liver congestion.

Conclusions:

LD₅₀ metam potassium= 1000 mg/kg bw. Metam potassium should be classified as harmful and assigned the symbol **Xn**, risk phrase **R22**.

GLP: yes

Guidelines: in agreement with Dir 96/54/EEC Annex IV B1 ter: test on kalium

Deviation from official protocol:

Material and Methods:

3 Wistar rats/sex after overnight fasting received a single oral dose of metam potassium (batch n° V814/2207KF, 54.7% w/w) at 200 mg (females and males) and 2000 mg/kg bw (3 G2 females). The test suspension was made just before dosing and homogeneity was maintained during dosing by constant mixing. The study is accepted.

B.6.2.2b Acute percutaneous toxicity with metam potassium (Annex IIA 5.2.2)

- **Acute dermal toxicity study (Acute toxic Class Method) with metam potassium aqueous solution in Wistar rats at 2000 mg/kg bw (Sulaiman, 2002)**

Findings:

There were no pre-terminal deaths. All the rats gained weight through the observation period and there were no toxic signs.

Erythema was observed in one male and in all female rats on day 3 and 4, post application. Erythema persisted along with scale formation was seen in all female rats on day 5 and continued in 2 females until day 6 followed by peeling/desquamation of skin on day 7.

The skin was normal on day 8 onwards. No abnormalities were seen at necropsy.

Conclusions:

Acute dermal LD 50 > 2000 mg/kg bw.

GLP: yes

Guidelines: in agreement with Dir 92/69/EEC the test is a limit test at 2000 mg/kg bw.

Material and Methods: 5 Wistar rats/sex were exposed dermal to 2000 mg/kg bw metam potassium aqueous solution (batch n° V814/2207KF, 54.7% w/w) applied undiluted on the hair clipped skin for 24 h. After exposure, the dressing was removed and skin washed with water, water + Teepol and again water.

The study is accepted.

B.6.2.3b Acute inhalation toxicity with metam potassium (Annex II A 5.2.3)

- **Acute inhalation toxicity study with metam potassium aqueous solution in Wistar rats at 2.98, 3.21 and 3.90 mg/L (Venugopala Rao, 2002)**

Findings:

Preliminary studies were conducted using undiluted metam to determine the atomisability and aerosol generation, selection of atomizer, concentration attained and pre-terminal deaths. At top dose of 2.85 mg/L, which is the maximal achievable concentration, two males and two female rats died.

Main study:

The pre-terminal deaths were 40, 70 and 90% in 2.98, 3.21 and 3.9 mg/L groups respectively and all deaths occurred either during exposure period or during the next day.

Toxic signs observed were lethargy, tremors, slight salivation and dyspnea in all the groups and ataxia was observed in one male rat exposed to 3.21 mg/L. These signs were seen predominantly on day 1 soon after exposure and on day 2 to 5.

Body weight: at mid and high dose, surviving rat lost weight at 8th day weighing but gained weight at terminal weighing and all the dead rats had reduced weight. At low dose, dead rats lost weight.

Necropsy: dead rats had lung congestion.

Conclusions:

LC₅₀ combined = 3.04 mg/L (≈ 820 mg/kg bw)

LC₅₀ male = 3.11 mg/L

LC₅₀ female = 3.03 mg/L

Metam potassium should be classified as harmful by inhalation and assigned the symbol **Xn** and risk phrase **R20**.

GLP: yes

Guidelines: not fully in agreement with Dir 92/69/EEC, 84/449, or OCDE 403 (1981).

Deviation from official protocol: relative humidity too high and temperature too high for 1 group.

Material and methods: 5 Wistar rats/sex/dose were exposed head and nose to an aerosol of metam potassium (batch n° V814/2207 KF, 54.4%) at 2.98, 3.21 and 3.90 mg/L for 4 hours.

group	Concentration in air (mg/L)	MMAD	% Respirable (<6µm)
1	2.98	1.30±0.88	99
2	3.21	1.19±0.78	99
3	3.90	1.10±0.64	99

The study is accepted.

B.6.2.4b Skin irritation with metam potassium (Annex II A 5.2.4)

No data.

B.6.2.5b Eye Irritation with metam potassium (Annex IIA 5.2.5)

- **Acute eye irritation/corrosion study with metam potassium aqueous solution in new Zealand white rabbits (Prakash, 2002)**

Findings:

In unwashed eyes:

<Score cornea>_{24+48+72h} = 0/0/0

<Score iris>_{24+48+72h} = 0/0/0

<Score conjunctivae redness>_{24+48+72h} = 0/0/0

<Score conjunctivae chemosis>_{24+48+72h} = 0/0/0

Conclusions:

Metam potassium is not irritating to eyes.

GLP: yes

Guidelines: in agreement with Dir 92/69/EEC.

Material and Methods: 0.1 ml of undiluted metam potassium (batch n° V814/2207KE, 54.7% w/w) was applied into the left eye of three New Zealand white rabbits

The study is accepted.

B.6.2.6b Skin sensitization with metam potassium (Annex IIA 5.2.6)

- **Metam potassium. Dermal sensitization study in guinea pigs (Magnusson-Kligman method) (Merkel, 2002)**

Findings:

Based on the results of the preliminary test, the concentration, which produced moderate irritation selected for intradermal induction, was a 5% w/w solution in distilled water and that which produced faint to moderate irritation selected for topical induction was a 25% w/w mixture in distilled water. For challenge, a 1% mixture in distilled water was used. Due to the presence of higher than expected scores following the challenge and first re-challenge, the dose concentration for the second re-challenge was reduced to 0.5%.

After topical induction, faint to severe erythema was noted for all sites one hour after patch removal. After challenge, moderate erythema was noted at one site 24 and 48 h after challenge. Eight other animals exhibited very faint (0.5) erythema at 24 and 48 h after challenge. After first re-challenge, faint erythema (1) was noted at two test sites 24 h after. Very faint erythema was noted at nine other sites. Very faint erythema persisted at six sites through 48 h. After the second re-challenge faint erythema was noted at two test sites 24 h and 48 h after re-challenge. Very faint erythema was noted at 10 other sites. Very faint erythema persisted at nine sites through 48 h.

Table B.6.2.6-1b: results of skin sensitization assay in guinea pigs with metam potassium.

Animal n°	Hours after patch removal scores					
	challenge		1 st Re challenge		2 nd Re challenge	
	24 h	48 h	24 h	48 h	24 h	48 h
1	0	0	0.5	0	0	0
2	0.5	0.5	0.5	0	0	0
3	0.5	0.5	0.5	0	0.5	0.5
4	0	0	0.5	0.5	0	0
5	0.5	0.5	0.5	0.5	0.5	1
6	0.5	0.5	0.5	0	1	0
7	0.5	0.5	0	0	0	0.5
8	0.5	0.5	0	0	0.5	0
9	0	0	0	0	0	0.5
10	0.5	0.5	0.5	0.5	0.5	0.5
11	0	0	0.5	0	0.5	0.5

Animal n °	Hours after patch removal scores					
	challenge		1 st Re challenge		2 nd Re challenge	
	24 h	48 h	24 h	48 h	24 h	48 h
12	2	2	0.5	0.5	0.5	1
13	0.5	0.5	0	0	1	0.5
14	0	0	0	0	0.5	0
15	0	0	0	0	0	0
16	0	0	0	0	0	0
17	0	0	1	0.5	0.5	0
18	0	0	1	0.5	0.5	0.5
19	0	0	0	0	0.5	0.5
20	0	0	0	0	0	0

Conclusions:

Based on these findings, metam potassium is not considered a contact sensitizer.

Guidelines: in agreement with Dir 96/54/EEC Annex IV C or 92/69-84/449 or OECD 406 (1981-92).

GLP: yes (no attest of competent authority)

Material and Methods: preliminary irritation screen: 12 guinea pigs Hartley albino were used to determine the appropriate concentrations of metam potassium to be used. Three concentrations were tested: 1%, 3% and 5% in water for the intradermal injection and a 50% v/v suspension of Freund's adjuvant in water. For topical application, the test substance was applied neat and at 75%, 50% or 25%. Prior to challenge phase, the fur was removed on the supra capsular area and test substance was applied at 50%, 25%, 12%, 9%, 6%, 3%, 1% w/w. Metam potassium (lot n° V814R/2207KF; PSL ref N° 020408-6H, 54.2%).

In the main study, 20 animals were used in the test group and 10 in the control group.

Main study:

Injection site n° Induction phase		Material injected
Left upper back	Right upper back	
1	1	50% v/v complete Freund's adjuvant mixture in distilled water
2	2	Test substance as a 5% w/w solution in distilled water
3	3	5% w/w suspension of test substance in complete Freund's adjuvant (50% in distilled water)
Topical induction		25% w/w a.i. in water
Challenge		1% w/w mixture of test substance in water: results inconclusive
1 st re-challenge		1% w/w in water: results inconclusive
2 nd re-challenge		0.5% w/w

For the control animals, active substance was not used. The study was validated using alpha-hexylcinnamaldehyde, technical grade, 85% as positive control.

The study is accepted.

B.6.2.1.c Acute oral toxicity with MITC (Annex IIA 5.2.1)

- **Report on the study of acute oral toxicity of the rat based on OECD and EPA (FIFRA) of MITC at 68.1, 100, 147, or 216 mg/kg bw (Kieczka and Kirsch, 1986)**

Findings:

Mortality: was noted at 147 mg/kg bw affecting 3 females and 1 males, occurring after day 1 and at 215 mg/kg bw where all animals died, starting after 1 hour exposure.

Clinical observations: unspecific signs of poisoning occurred, like dyspnea, apathy, staggering, paresis, twitching, piloerection, exsiccosis, salivation and poor general state.

Necropsy: in death animals, general congestive hyperemia; some animals were cadaverous and diagnosis was not possible

Conclusions:

LD50 MITC = 147mg/kg bw. MITC should be classified as toxic and assigned the symbol **T**, risk phrase **R25**.

GLP: yes (no attest of competent authority)

Guidelines: Not fully in agreement with Dir 92/69/EEC, 84/449, or OCDE 401 (1987-81): this is a test with MITC.

Deviation from official protocol: Individual data not included in the study, clinical signs reported/group without indication of the number of affected rats.

Material and Methods:

5 Wistar rats/sex/dose received after overnight fasting by gavage a single dose of MITC (lot n° 6205 MK, 98%) at 68.1, 100, 147 or 216 mg/kg bw. MITC was emulsified in olive oil after heating to about 40°C. The study is accepted.

B.6.2.2c Acute percutaneous toxicity with MITC (Annex IIA 5.2.2)

- **Report on the study of the acute dermal toxicity of the rat based on OECD and EPA (PIFRA) of MITC at 215, 1000, 1470 and 2150 mg/kg bw (Kirsch and Hildebrand, 1987)**

Findings:

Mortality occurred at 1000, 1470 and 2150 mg/kg bw. Toxic signs were dyspnea, apathy, staggering, tremors and poor general state. Necropsy findings in animals that died were general congestion, bloody ulcerations in the glandular stomach. As local findings erythema, edema and scaling were observed.

Conclusions:

Acute combined dermal LD50 = 1290 mg/kg bw. MITC should be classified as harmful in contact with skin and assigned the symbol Xn and risk phrase R21.

GLP: yes (no attest of competent authority)

Guidelines: not fully in agreement with Dir 92/69/EEC: the test is performed with MITC.

Deviation from official protocol: individual data were not included in the dossier; clinical signs are reported per group without indication of the number of affected rats.

Material and Methods:

5 Wistar rats/sex/dose were exposed to 215, 1000, 1470 and 2150 mg/kg bw MITC (lot n° 6205 MK; 98%) by application to skin under semi-occlusive dressing for 24 h. after removal of the dressing, the site was rinsed with warm water.

The study is accepted.

B.6.2.3c Acute inhalation toxicity with MITC (Annex IIA 5.2.3)

- **Methyl isothiocyanate: Acute inhalation toxicity in rats, 4 hour exposure at 0.786, 1.64, 0.282, 0.496, 0.628, and 0.540 g/m³ (Jackson et al, 1981)**

Findings:

Mortality: occurred within 2 days of exposure except one animal dying the third day.

Table B.6.2.3-1c: mortality in rats after MITC inhalation

Dose (g/m ³)	Death	
	M	F
0	0/5	0/5
0.282	0/5	0/5
0.496	1/5	1/5
0.540	3/5	3/5
0.628	5/5	5/5
0.786	5/5	5/5
1.64	5/5	5/5

Clinical signs noted during exposure were consistent with marked irritation and included closure of eyes, lachrymation and peripheral vasodilatation in all exposed rats and excessive salivation and adoption of hunched posture. During the observation period, peripheral vasodilatation and wet jaws due to excessive salivation were observed. Surviving animals had swollen abdomens, which persisted throughout the first day of observation. Animals had rales during days 3-4 of observations. Opacity of eyes was seen in all surviving rats at 0.786, 0.628 and 0.570 g/m³. Animals presented poor grooming, staining around head or snout and lethargy.

Body weight loss was seen excepting group 0.282 g/m³ and females in group 0.496 g/m³.

Food and water consumption was reduced following exposure but recovered rapidly.

Lung weight/body weight ratio was increased in dead animals.

Necropsy findings were confined to lungs (congestion) and GI tract (distended with gas) of rats that died.

Microscopy showed lung lesions and liver focal necrosis.

Conclusions:

LC50 = 0.54 g/m³ for both sexes (= 0.54 mg/L $\cong$ 145 mg/kg bw)

MITC should be classified as toxic by inhalation and assigned the symbol T and risk phrase R23. RMS proposes to add an additional classification as Xi, R37 related to lung lesions observed in animals dying after exposure.

GLP: yes (no attest of competent authority)

Guidelines: not fully in agreement with Dir 92/69/EEC, 84/449, or OCDE 403 (1981).

Deviation from official protocol: temperature is slightly too high. Incomplete raw data for clinical signs and food consumption.

Material and Methods: 5 CD rats/sex/dose were exposed whole body to MITC (98%) at 0.282, 0.496, 0.540, 0.628, 0.786 and 1.64 g/m³ for 4 hours.

The study is accepted.

B.6.2.4c Skin irritation with MITC (Annex IIA 5.2.4)

- **Report on the study of the acute dermal irritation/corrosivity to the intact dorsal skin of the white rabbit based on OECD and EPA (FIFRA) of MITC (Kirsch and Kieczka, 1986)**

Findings:

<Score erythema> 30 min + 48 h + 72 h = 4

<Score edema> 30 min + 48 h + 72 h = 3.8

After application, severe signs of irritation were observed which persisted up to 72 h. At this time superficial necrosis were seen in all animals. As reversibility could not be expected, the study was terminated after 72 h.

Conclusions:

MITC is corrosive to the skin. The symbol C and the risk phrase R34 should be assigned.

GLP: yes (no attest of competent authority)

Guidelines: not fully in agreement with Dir 92/69/EEC or 84/449/EEC or OECD 404(1992-81)

Deviation from official protocol: test on MITC: scores were noted at 24, 48 and 72 h after starting exposure and not at the end of exposure period. The study is stopped after 72 h anticipating reversibility. Skin was partly scarified according to the summary but this is not mentioned in the experimental protocol. Scoring criteria are not described

Material and Methods: 0.5 ml of MITC (lot n° 6205 MK, 98%) was applied to the intact and scarified dorsal skin of 3 White Vienna rabbits/sex under semi-occlusive dressing for 4 h after which the dressing was removed and skin was washed with Lutrol and water.

The study is accepted.

B.6.2.5c Eye Irritation with MITC (Annex IIA 5.2.5)

No data not required: MITC is corrosive for the skin, therefore no study is required.

B.6.2.6c Skin sensitization with MITC (Annex IIA 5.2.6)

- **Report on the M&K test for the sensitizing potential of MITC in guinea pig (Kieczka and Kirsch, 1986)**

Findings:

After intradermal induction, distinct erythema and edema were observed at the injection sites of the control animals and the test animals, which were applied with Freund's adjuvant/water. Test substances formulations in olive oil in Freund's adjuvant/water caused necrotic skin changes and distinct edema in the test group. The control animals, which were applied with olive oil, exhibited distinct erythema.

After the percutaneous induction, necrotic skin changes and distinct edema were observed in the test group. Olive oil, which was applied to the control animals, caused incrustation (partially open) in addition to distinct erythema and edema. The number of animals with skin findings after the first challenge and after the second challenge is reported in table B.6.2.6-2.

After the first challenge, 7/20 test animals exhibited slight erythema, 4/20 test animals showed distinct erythema in addition to slight edema and in 1/20 animals distinct erythema was observed. Control group 1 did not show any skin changes.

After the second challenge, 7/20 test animals showed slight erythema, 2/20 had distinct erythema in addition to slight edema and in 4/20 test animals distinct erythema was seen. Control groups 1 and 2 did not show any skin changes.

Olive oil, applied as vehicle, did not cause any skin reactions after the first and the second challenge either in the control animals or in the test group.

MITC has a sensitizing effect on the skin of the guinea pig.

Table B.6.2.6-1c: skin findings in guinea pigs exposed to MITC in M&K test.

	Number of + response/number tested animals 48 h after application			
	1 st challenge		2 nd challenge	
	0.5% in olive oil	Olive oil unchanged	0.5% in olive oil	Olive oil unchanged
Control group 1	0/10	0/10	0/10	0/10
Control group 2	No application of test substance	0/10	0/10	0/10
Test group	12/20	0/20	13/20	0/20

Conclusions:

MITC should be considered as a skin sensitizer. The symbol Xi and risk phrase R43 should be assigned.

Guidelines: not fully in agreement with Dir 96/54/EEC Annex IV C or 92/69-84/449 or OECD 406 (1981-92). Test with MITC

Deviation from official protocol: concentrations used for induction are too high and induce tissue necrosis. Pretest results not reported.

GLP: only attest of GLP/OAU

Material and Methods:

MITC (batch n° 6205MK; 98%) was tested in 30 Pirbright White Dunkin Hartley female guinea pigs (10 controls, 20 exposed). In a preliminary test after 24 h with percutaneous occlusive applications within 96 h, the minimum irritant concentration was found to be a 2% emulsion in olive oil and the maximum non-irritant concentration a 1% emulsion in olive oil. However, because this concentration of 1% was very close to the 2% test substance concentration, which causes distinct irritation effects in ¼ animals in a pretest with two applications, a 0.5% emulsion in olive oil was used for challenge. The study is accepted.

The following concentrations were selected based on the pretests:

Intradermal induction	2% in olive oil in Freund's adjuvant/water (1:1)
Percutaneous induction	2% in olive oil
1 st challenge	0.5% in olive oil
2 nd challenge	0.5% in olive oil

B.6.2.7 Summary of acute toxicity including irritancy and skin sensitization (Annex IIA 5.2)

Metam breaks down to MITC, which is the ultimate toxicant. The gaseous decomposition of metam sodium into MITC under acidic conditions justifies the need for a R31.

Acute oral exposure to metam or MITC led to pilo-erection, hunched posture, abnormal gait, lethargy, decreased respiration rate, ptosis, and pallor of extremities, increased salivation, and clonic convulsions. Metam and MITC should be classified as harmful or toxic by ingestion, respectively.

Hypothermia was reported after metam sodium dermal exposure while quite similar signs as those reported after oral exposure were seen after MITC dermal exposure. MITC should be classified as harmful by skin exposure.

After whole body inhalation exposure to metam, signs consistent with exposure to an irritant and toxic aerosol were noted. Histopathology of the lungs showed bronchiolar epithelial necrosis with inflammatory exudates.

Classification as harmful by inhalation and R20 is required according to this study. A nose and head only inhalation exposure was realized where reddish discharges from nose and eyes were reported and animals had high stepping gait. According to the results of this study, metam should not be classified for inhalation toxicity.

Clinical signs reported after exposure to MITC by inhalation were quite similar to those seen after metam. MITC requires a classification as toxic by inhalation (whole body inhalation study).

Metam and MITC were corrosive in the skin irritation test. No irritation effects were seen in the eye irritation test. Skin sensitization potential was seen for metam sodium and for MITC.

Table B.6.2.7-1: Summary of acute toxicity of Metam sodium

Type of test Test species	Test substance purity	Results	References
Acute oral, rat	Batch n°? 510 g/L	Combined: 896 mg/kg bw harmful Xn, R22	Baldrick and Healing, 1991a
Acute dermal, rat	Batch n°? 510 g/L	LD50 > 2000 mg/kg bw	Baldrick and Healing, 1991b
Acute inhalation, rat whole body	Batch n°? 510 g/L	LC50 combined = 2.54 mg/L Xn, R20	Jackson and Hardy, 1992
Acute inhalation, rat head and nose	42.2%, batch ZH 130585	LC50 > 6.8 mg/L	Gelbke and Klimisch, 1986
Acute skin irritation, rabbit	Batch n°? 510 g/L	Corrosive : C, R34	Ligget and McRae, 1991a
Acute eye irritation, rabbit	Batch n°? 510 g/L	Non irritant	Ligget and McRae, 1991b
Skin sensitization- M&K test	Batch n°? 510 g/L	Skin sensitizer, R43	Parcell and Danton, 1991

Table B.6.2.7-2: Summary of acute toxicity of Metam potassium

Type of test Test species	Test substance purity	Results	References
Acute oral, rat	batch n° V814/2207KF, 54.7% w/w	1000 mg/kg bw harmful Xn, R22	Sulaiman, 2004
Acute dermal, rat	V814/2207KF, 54.7% w/w	LD50 > 2000 mg/kg bw	Sulaiman, 2002
Acute inhalation, rat head and nose	batch n° V814/2207KF, 54.7% w/w	LC50 combined = 3.04 mg/L Xn, R20	Venugopola Rao, 2002
Acute skin irritation, rabbit Metam sodium	Batch n°? 510 g/L	Corrosive : C, R34	Ligget and McRae, 1991a
Acute eye irritation, rabbit	batch n° V814/2207KF, 54.7% w/w	Non irritant	Prakash, 2002
Skin sensitization- M&K test	batch n°	Not sensitizer	Merkel, 2002

Type of test Test species	Test substance purity	Results	References
	V814/2207KF, 54.2% w/w		

Table B.6.2.7-3: Summary of acute toxicity of MITC

Type of test Test species	Test substance purity	Results	References
Acute oral, rat	MITC; lot n° 6205 MK, 98%	Combined: 147 mg/kg bw. Toxic T, R25	Kieczka and Kirsch, 1986
Acute dermal, rat	Lot n° 6205 MK, 98%	1290 mg/kg bw Xn, R21	Kirsch and Hildebrand, 1987
Acute inhalation, rat whole body	98%	LC50 = 0.54 mg/L T, R23 Xi, R37	Jackson et al, 1981
Acute skin irritation, rabbit	Lot n° 6205 MK, 98%	Corrosive : C, R34	Kirsch and Kieczka, 1986
Acute eye irritation study		No study is required.	
Skin sensitization- M&K test	Batch n°, 6205MK; 98%	Skin sensitizer; R43	Kieczka and Kirsch, 1986

B.6.3a Short-term toxicity with metam sodium (Annex IIA 5.3)**B.6.3.1a Oral 28-day toxicity (Annex IIA 5.3.1)**

The studies reported here are both range-finding studies in order to set dose levels for a subsequent 90-day study.

- **Metam Sodium: pilot drinking water study in mice (Whiles, 1990)**

Metam-sodium in aqueous solution produces MITC. Therefore, drinking water administration of metam-sodium to laboratory animals may provide data relevant to the toxicological evaluation of MITC in air.

Findings:

There were no adverse clinical signs related to treatment.

Body weight of older male mice reduced at 0.62 mg/ml during the first four days of the study, recovering later but they still had a slight body weight loss after 8 days. After 0.35 mg/ml, an initial loss of bodyweight on day 1 was noted and then showed some recovery. Females had an overall loss at top dose. At 0.35 mg/ml and in control group, there was no growth.

Although all groups showed a body weight loss for the first day, the younger mice of both sexes given 0.35 or 0.62 mg/ml gain weight over the six-day period.

Water consumption: there was a dose-related reduction in water consumption of the older and younger mice.

Food consumption: there was no consistent effect in older and younger mice. There was however, evidence of a slight reduction in food consumption of younger females at top dose.

There were no macroscopic findings suggesting a treatment-related effect (table B.6.3.1-1a).

Table B.6.3.1-1a: Range-finding study in older and younger mice.

Endpoints	Doses					
	0		0.35 mg/ml		0.62 mg/ml	
	M	F	M	F	M	F
Body weight day 8 (g)						
Older mice	24.6	20.8	24.8	21.0	21.8	20.6
Younger mice	-	-	18.0	14.2	17.6	13.8
Water consumption (ml/day) :						
Older mice	3.4	3.8	2.9	2.4	2.2	2.2
Younger mice	-	-	3.4	2.3	2.8	1.6
Food consumption g/day						
Older mice	4.2	4.5	3.6	4.6	5.6	4.6
Younger mice	-	-	4.4	4.2	4.2	2.4
Compound intake (mg/kg bw/d)			41	40	62.6	66.2

Conclusions:

It is concluded that metam sodium up to a concentration of 0.62 mg/ml is sustainable as the highest dose for a mouse study of 90 days.

GLP: no attest of GLP, but an attest of authenticity was provided.

Guidelines: not fully in agreement with Dir 96/54/EEC Annex IV D or 92/69-84/449 or OECD 407 (1995-1981).

Deviation from official protocol: The duration of the test was of 6 days on young animals and of 8 days on older animals; only two concentrations were tested; blood analysis, organ weight and histopathology not performed.

Material and Methods: 15 C57/BL/10JfAP/Alpk mice/sex/dose (70 days of age) received for 6 days, in drinking water at 0, 0.35 or 0.62 mg/ml, metam sodium (525.54 g/L) which was added to an appropriate amount of water and mixed thoroughly and pH was adjusted to 9.0. An additional group of 10 C57/BL/10JfAP/Alpk mice/sex/dose, which were younger (31-33 days of age), received 0.35 or 0.62 mg/ml metam sodium in drinking water for 6 days. Younger animals started 2 days after older animals.

The dosing preparation was prepared fresh, daily.

The study is accepted.

- **Metam sodium: 21 day drinking water study in rats (Cave, 1991)**

Findings:

The initial dose level of 1, 3 or 6mg/ml was reduced, as the rats did not drink water containing too high concentrations of metam. The rats of the higher dose level of 0.7 mg/ml did not drink the water. Lower dose levels of 0.1 and 0.3 mg/ml were acceptable and the rats drank this water for 21 days, but water consumption was reduced in a dose dependent fashion. Food consumption was reduced, resulting in reduced body weight gain. There was no dose related effect on organ weight or organ weight: body weight ratio (table B.6.3.1-2a).

Table B.6.3.1-2a: Range-finding study in rats.

Endpoints/dose	0		0.1mg/ml		0.3mg/ml		0.7mg/ml	
	M	F	M	F	M	F	M	F
Body weight day 18 (g)	78.6	35.8	76.6	33.4	61.8*	23.2		
Day 30	129.8	56.6	121.2	51	111.4*	46.4		
Water consumption								
Day 7	32.6	21.6	23.2	14.8	11.0	9.4	3.9	3.4
Day 18	29.6	24.8	24	19.6	22.4	15	-	-
Day 30	34.2	30.4	28.6	21.8	22.8	13.4	-	-
Food consumption (g)								
Week 2	26.7	19.5	27.1	19.9	23.9	17.2		
Week 3	27.7	18.8	28	19.2	25.3	16.8		
Week 4	27.9	19.5	28.3	19.8	26.1	17.9		
Organ weight :bw ratio								
Brain	0.566	0.811	0.57	0.812	0.621	0.837	0.55	0.866
Kidney	0.764	0.765	0.721	0.857	0.808	0.865*	0.729	0.782
Liver	4.848	4.148	4.603	4.398	4.853	4.023	4.554	4.049
Spleen	0.305	0.278	0.278	0.263	0.301	0.313	0.321	0.321
Testis	0.853	-	0.908	-	0.928*	-	0.905	-

*statistically significantly different from control.

Conclusion:

Metam sodium included in drinking water at levels of 0.1 or 0.3 mg/ml caused a decreased bodyweight gain and food consumption, which could be related to the reduced water consumption. The maximum tolerated level of metam sodium in drinking water is below 0.7 mg/ml. For the 90-day study dose, levels of 0.5 mg/ml should be the maximum tolerated dose and the low level should be 0.02 mg/ml.

GLP: no

Guidelines: not fully in agreement with Dir 96/54/EEC Annex IV D or 92/69-84/449 or OECD 407 (1995-1981).

Deviation from official protocol: Absence of individual data; very brief test and result description.

Material and Methods: 20 Alpk: ApfSD rats/sex/dose received metam sodium (batch n° 11877-9-1, 32.8 g/L) dissolved in water at pH 9 for 21 days. The dosing solution was prepared daily. The initial inclusion level of metam sodium was 1, 3, or 6 mg/ml. As the rats did not drink the water, the dose levels were reduced to zero, 0.1, 0.3 and 0.7 mg/ml. rats in top dose group did not drink water.

The study is accepted.

B.6.3.2.1a Oral 90-day toxicity (Annex IIA 5.3.2)

- **Metam Sodium: 90-day drinking water study in mice at 0.018, 0.088, 0.35 and 0.62 mg/ml with a 28 day interim kill (Whiles, 1991)**

Findings:

The mean doses were stated to be 0, 2.7, 11.7, 52.5, or 78.7 mg/kg/day for males and 0, 3.6, 15.2, 55.4, or 83.8 mg/kg/day for females. However, due to the chemical instability of metam sodium in drinking water, the nominal doses of metam sodium received could only be considered approximations. Analysis of the stability of metam sodium in drinking water bottles indicated that, depending on the concentration, 15-70.9% of the chemical was lost by 24 hours. The mean dose of metam sodium actually received would then have been lower and could have been as low as 0, 0.79, 4.48, 36.05 and 60.36 mg/kg/day for males and 0, 1.05, 5.82, 38.12 and 64.27 mg/kg/day for females (these values were extracted from the California EPA (2004) who calculated from the 24- hr decay data measured on a single occasion).

Mortality was not compound-related and affected two females at 0.35mg/ml.

Clinical signs: there were no compound-related signs.

The other histopathological findings, after 90 days of treatment, of significance were cystitis, mucosal hyperplasia and the presence of eosinophilic granules in transitional epithelial cells of the urinary bladder of animals given 0.35 or 0.62 mg/ml and the presence of eosinophilic granules in animals given 0.088 mg/ml. A consistent dose-relationship in terms of incidence or severity was not shown.

Endpoints/dose	0 mg/ml		0.018		0.088		0.35		0.62 mg/ml	
	M	F	M	F	M	F	M	F	M	F
Compound intake mg/kg bw/d	0	0	2.7	3.6	11.7	15.2	52.4	55.4	78.7	83.8
Actual compound intake	0	0	0.79	1.05	4.48	5.82	36.05	38.12	60.36	64.27
Mortality:		1						2		
Body weight:							↓9%*	↓8%	↓13%*	↓11%*
Bw gain Wk 13							↓26%		↓19%	↓10%
Food consumption: week 13							↓4%	↓10%	↓7%*	↓18%
Water consumption										
Week 4					↓11% *	↓18% *	↓15% *	↓30%*	↓31%*	↓38%*
Week 1- 13					↓10%	↓20%	↓4%	↓29%*	↓21%*	↓40%*
Interim sacrifice: day 28										
Relative organ weight										
Liver							↑21% *	↑15%*	↑23%*	↑21%*
Macroscopy: n° affected animals/total examined										
Liver accentuated lobular pattern:							1/5	3/5	1/5	2/5
Microscopy: n° affected animals/total examined										
Kidney tubular basophilia										1/5
Testis tubular atrophy:									2/5	
Liver centrilobular hypertrophy:									1/5	
Liver inflammatory cell infiltration:										2/5
Stomach cystic gland:										1/5
Terminal kill										
Hematology:										
Hb						↓4%*	↓3%*	↓6%*	↓5%*	↓7%*
RT						↓4%*	↓5%	↓8%*	↓3%*	↓7%*
RBCcount						↓4%*	↓3%*	↓10%*	↓6%*	↓10%*
Relative organ weight at terminal sacrifice										

Endpoints/dose	0 mg/ml		0.018		0.088		0.35		0.62 mg/ml	
	M	F	M	F	M	F	M	F	M	F
liver					↑8%*	↑5%*	↑28%*	↑26%*	↑26%*	↑23%*
kidney							↑10%*	↑10%*	↑9%*	↑11%*
Macroscopy: n° affected animals/total examined										
Liver accentuated lobular pattern:							1/10	2/10	2/10	0/10
Spleen black area		0/10		0/10	1/10	1/10	0/10	1/10	1/10	2/10
Microscopy: n° affected animals/10 mice										
Bladder:										
mucosal hyperplasia	0	0	0	0	0	0	10		9	8
Cystitis	0	0	0	0	0	0	10	1	8	0
Eosinophilic granules in epithelium	0	0	0	0	10	10	7	8	10	10

*statistically significantly different from control

Conclusion:

The administration of metam sodium in drinking water of mice for up to 90 consecutive days produced general toxicity at dose levels of 0.35 or 0.62 mg/ml characterized by impaired body weight gain, slightly reduced food consumption and minor hematological changes. At dose levels of 0.088, 0.35 and 0.62 mg/ml, histopathological changes, possibly because of compound, were apparent in the bladder. The NOAEL = 0.018 mg/ml = 0.79 mg/kg bw/d.

GLP: yes, (no attest of competent authority)

Guidelines: not fully in agreement with Dir 87/302/EEC.

Deviation from official protocol: ophthalmoscopy and blood chemistry were not performed

Material and Methods: 15 C57/BL/10JfAP/Alpk mice/sex/dose received for 29 or 90 days, via drinking water at 0, 0.018, 0.088, 0.35 or 0.62 mg/ml, metam sodium (ref n° Y06930/007 and Y06930/008, 525.54 g/L) which was added to an appropriate amount of water and mixed thoroughly and pH was adjusted to 9.0. Chemical stability of metam sodium in water purified by reverse osmosis showed a significant reduction over 24 h periods a fresh dosing preparations were prepared daily. Mean dose of metam sodium received were calculated from the nominal concentrations using measurements of the maximum 24-hour degradation rates in water bottles. The degradation rates, which were determined in an assay performed in parallel with the study, were highly dependent on the initial metam sodium concentration in the water bottle. If that concentration was less than 0.1 mg/ml, approximately 60-70% of the metam was shown to disappear after 24 hours. If the concentration was more than 0.1 mg/ml, degradations of 32% or less occurred within that time. While it was stated in the study that the drinking water containing the test article was changed daily, the time of the change was not provided, nor was there any indication as to how long after each change the water was consumed by the mice. At doses of 0.018 and 0.088 mg/ml, the only assurance was that the animals were drinking water containing between 29.1% and 100% of the initial concentration.

When combined with the lack of sub-24-hour degradation curves, there was no way to accurately gauge the quantity of metam that the animals. It was therefore assumed that maximum degradation had occurred by the time the mice drank the water. Thus, at the nominal concentration of 0.018 mg/ml, the initial analyzed concentration was 0.022 mg/ml. After 24 hours, the metam concentration had declined to 0.0063 mg/ml, or 29.1% of the initial concentration. Using mean data provided in the study, it was stated that the metam sodium consumption at that dose was 2.7 mg/kg/day. This value was derived by the study authors using only the initial metam concentration, the body weights appropriate to that point in the study, and the measured water consumption rates. Assuming maximum degradation, the "actual" metam consumption was calculated to be $0.291 \times 2.7 = 0.79$ mg/kg/day. At 0.088 mg/ml, the initial analyzed concentration was 0.081 mg/ml. After 24 hours, the concentration was 0.031 mg/ml, or 38.3% of the initial concentration. The stated consumption at that dose was 11.71 mg/kg/day, making the "actual" consumption $0.383 \times 11.71 = 4.48$ mg/kg/day.

Statistical analysis: analysis of variance/covariance for body weights, water consumption, food consumption and hematology and organ weights. Differences from control were tested by comparing each group using a two-sided Student's test.

The study is accepted.

- metam sodium: 90 day drinking water study in rats at 0.018, 0.089, 0.443 mg/ml (Allen, 1991)

Findings:

The mean doses were stated to be 0, 1.7, 8.1, or 26.9 mg/kg/day for males and 0, 2.5, 9.6, and 30.6 mg/kg/day for females.

However, due to the instability of metam sodium in drinking water, the mean dose of metam sodium actually received could be 0, 0.49, 3.10 and 18.51 mg/kg/day for males and 0, 0.73, 3.68 and 21.05 mg/kg/day for females (as calculated by the California EPA, 2204).

Mortality: affected one female at top dose on day 5 and was not considered compound related.

Clinical signs: top dose females had thin appearance, which was reflected in the lower body weight of these animals.

Body weight, food consumption, water consumption: was reduced at top dose to both sexes and to females at 0.089 mg/ml.

Ophthalmoscopy: there were no changes attributable to metam.

Hematology: very small changes in RBC counts achieving statistical significance at all dose levels and in hematocrit (except in males at 0.018 mg/ml) were within the normal range.

Urinalysis: the alteration in urine output from rats of both sexes at top dose, particularly reduced volume, increased specific gravity and increased renal epithelial cell excretion were considered to reflect reduced water consumption. In the absence of histopathological damage to the kidneys, this increase in renal epithelial cell excretion almost certainly reflects the reduction in urinary volume output and consequent increase in osmolarity of the urine.

Blood chemistry: reduced values for several parameters were observed at top dose including phosphorus, AST, ALT and ALP reflecting probably the poor nutritional status of the animals. A small increase in triglycerides was seen in females at top dose and plasma urea was increased in this group.

Organ weight: small increase in relative kidney weight was statistically significant in females at top dose.

Histopathological changes were essentially confined to the nasal passages of both sexes, which received 0.443 mg/ml. The change was of greater incidence and severity in females. This change was specific to the olfactory epithelium in the posterior region of the nasal passages and was characterized by prominent and/or vacuolated Bowman's glands or ducts with disorganization and vacuolation of the olfactory epithelium. This lesion is unusual in both nature and location. No changes were seen in the respiratory epithelium.

The company proposed that the lesion was considered to reflect a systemic effect rather than incidental inhalation of the test substance because the posterior nasal cavity was reported to be more severely affected than the anterior. Another possible explanation for the lesions, also based on their location, is induction by exposure to the metam metabolites during exhalation.

From the open literature, in rat, the nasal cavity is a complex structure with regions lined by four distinct types of epithelium:

- The region of stratified squamous epithelium includes the nostrils, vestibule, anterior ventral meatus and incisive duct. This epithelium is resistant to the effects of inhaled toxicants.
- The region of transitional epithelium includes the anterior lateral wall and the anterior tips and lateral surfaces of the maxillary and nasal turbinate.
- The pseudo stratified ciliated respiratory epithelial region includes the nasal septum and an area between the transitional epithelium and olfactory region.
- The olfactory epithelium covers approximately 50% of the nasal cavity surface area and lines the posterior dorsal septum, the middle and posterior dorsal medial meatus and the ethmoid turbinate. Olfactory epithelium is comprised of olfactory sensory neurons, sustentacular cells, and basal cells. Beneath the olfactory mucosa is the *lamina propria* that contains the Bowman's glands that express secretory proteins as well as phase I and phase II biotransformation enzymes. Olfactory epithelium contains high levels of glutathione transferase (Brittebo, 1997). Concentrations of certain cytochrome P450 isozymes in the olfactory mucosa of monkeys have been reported to be approximately twice those in the olfactory mucosa of rats (Dahl, 1986).

Olfactory epithelium usually tends to be more sensitive epithelium to toxicants that need metabolic activation (Harkema, 1990). No such an organ specific effect was seen in mice, the species difference in susceptibility was also noted for other toxicants, and the mechanisms for species differences are not clear.

Table B.6.3.2.1-2a: 90-day drinking study in rats.

Endpoints/dose	0		0.018		0.089 mg/ml		0.443 mg/ml	
	M	F	M	F	M	F	M	F
Mortality								1
Compound intake mg/kg bw/d	0	0	1.7	2.5	8.1	9.3	26.9	30.6
Actual compound intake mg/kg bw/d	0	0	0.49	0.73	3.1	3.68	18.51	21.05

Endpoints/dose	0		0.018		0.089 mg/ml		0.443 mg/ml	
	M	F	M	F	M	F	M	F
Body weight						↓3-4% * from week 5-14	↓19-23% * from week 1-14	↓21-23% * from week 1-14
Food consumption						↓6%* week 4	↓37-16% * from week 1-13	↓47-20%* from week 1-13
Water consumption						↓32%* from week 1-9	↓65-47%* from week 1-13	↓72-62% * from week 1-14
Hematology								
hematocrit					↓4% *	↓3% *	↓4% *	↓3% *
RBCcount				↓4% *	↓5% *	↓4% *	↓6% *	↓4% *
Blood chemistry								
Glucose							↓13% *	
ALP							↓16% *	
ALT							↓13% *	
AST							↓15% *	
Plasma urea								↑54% *
Plasma cholesterol								↓10% *
Plasma triglycerides								↑33% *
Plasma sodium								↑0.99% *
Plasma phosphorus								↓12.5% *
Plasma chloride								↑2.5% *
Urinalysis								
Volume							↓50% *	↓35.5% *
Specific gravity							↑1.25% *	↑1.1% *
pH							↓6% *	
Protein							↓25% *	
Organ weight:								
Kidney relative						↑6%	↑6%	↑16% *
Liver relative						↑10% *		↑13%
n° affected rats								
Urinary sediment:								
Renal epithelial cells:	1	0	0	0	2	2	10	8
Red blood cells	0	0	1	1	1	1	0	3
White blood cells	0	0	2	1	2	1	0	3
Microscopy: terminal								
kidney								
Tubular basophilia	1/12	0/12	0/12	0/12	0/12	0/12	2/12	3/12
Nasal cavity: Olfactory epithelium								
disorganization	0/12	0/12	0/12	0/12	0/12	0/12	2/12	10/11
Prominent/vacuolated Bowman glands/duct	0/12	0/12	0/12	0/12	0/12	0/12	5/12	11/11
vacuolation	0/12	0/12	0/12	0/12	0/12	0/12	2/12	11/11
necrosis	0/12	0/12	0/12	0/12	0/12	0/12	0/12	1/11

*Statistically significantly different from control.

Conclusion:

a NOAEL can be set at 0.018 mg/ml (= 0.49 mg/kg bw/d) taking into account the decreased body weight resulting from reduced water consumption altering food consumption.

GLP: yes, but no attest of competent authority

Guidelines: not fully in agreement with Dir 87/302 or OECD 408 (1998-1981).

Deviation from official protocol: limited brain histopathology.

Material and Methods: 12 Alpk: ApfSD rats/sex/dose received metam sodium (batch n° Y06930/007 and Y03930/008; 525.54 g/L) in drinking water at 0, 0.018, 0.089 or 0.443 mg/ml for 90 days. Drinking water preparations were purified by reverse osmosis with pH adjusted to nine. Preparations were made fresh each day. Instability was evident in all formulations, with the lowest concentrations showing the largest reductions. The mean doses were stated to be 0, 1.7, 8.1, or 26.9 mg/kg/day for males and 0, 2.5, 9.6, and 30.6 mg/kg/day for females. However, according to the California EPA (2004) due to the instability of metam sodium in drinking water, the nominal doses of metam sodium received could only be considered approximations of the actual internal dose. Analysis of metam sodium stability in drinking water bottles indicated that, depending on the concentration, 15.0-70.9% of the chemical was lost by 24 hours. The mean dose of metam sodium actually received would then have been lower and could have been as low as 0, 0.49, 3.10 and 18.51 mg/kg/day for males and 0, 0.73, 3.68 and 21.05 mg/kg/day for females (these values were calculated from the 24-hr decay data measured on a single occasion). These values were calculated for drinking water studies from the nominal concentrations using measurements of the maximum 24-hour degradation rates in water bottles. The degradation rates, which were determined in an assay performed in parallel, were highly dependent on the initial metam sodium concentration in the water bottle. If that concentration was less than 0.1 mg/ml, approximately 60-70% of the metam was shown to disappear after 24 hours. If the concentration was more than 0.1 mg/ml, degradations of 32% or less occurred within that period. When combined with the lack of sub-24-hour degradation curves, there was no way to accurately gauge the quantity of metam that the animals consumed.

Statistical analysis: analysis of variance/covariance for body weights, water consumption, food consumption and hematology and organ weights. Differences from control were tested by comparing each group using a two-sided Student's test. The study is accepted. The study is accepted.

B.6.3.2.2a Oral 90-day toxicity (dog) (Annex IIA 5.3.2)

- metam sodium: 90-day oral dosing study in dogs, at 1, 5, 10 mg/kg bw/d (Brammer, 1992)

Findings:

Mortality: Two dogs were killed due to adverse clinical effects, which were compound-related.

Clinical signs: the most significant findings occurred in the last weeks of dosing at 10 mg/kg bw/d. Thinness and clinical signs of jaundice were observed in the two dogs killed inter-currently and in one male, which survived to scheduled termination. Salivation was present regularly. Some animals had regurgitation after dosing.

Body weight: loss associated with inappetence was apparent at top dose in the last few weeks of the study. The female group did not gain weight in the first week of the study and the rate of weight gain thereafter was less than the control without reaching statistical significance.

Food consumption: at top dose, animals left food towards the end of the study. At 5 mg/kg bw/d, one male left food in the last week of the study.

Hematology: clotting time (Kaolin-cephalin times) was slightly raised in males throughout the study and in females at week 4 (19%) and eight (11%) in the 10 mg/kg bw/d group. Protrombin times were slightly raised at week 13 in both male and females at top dose. Red blood cell indices (MCV, MCH and MCHC) were changed. Lymphocyte changes in males were inconsistent. Increase in monocytes was seen. These changes are considered secondary to the hepatotoxicity present at 5 and 10 mg/kg bw/d.

Blood chemistry: Triglycerides were increased in week 4 for males at 5 and 10 mg/kg bw/d and for females at 5 mg/kg bw/d and calcium in males at 5 and 10 mg/kg bw/day in week 4. Reduction in glucose and cholesterol were seen in males at the end of the study.

At the end of the study, at 10 mg/kg bw/d, ALT, AST, ALP and GGT, and total bilirubin were increased; males were more affected than females.

At 5 mg/kg bw/d, all dogs were affected to some extent: some increases in ALT were seen in males by the end of the study and ¾ dogs had increased ALP and AST. Females showed small increases in ALT from week 10 and 1 female showed increased ALP and AST in week 13.

At 1 mg/kg bw/d, one female showed small increases in ALT from week 4-12 with moderate increase in ALT and AST at week 13. Creatine kinase activities were increased in week 10-13 in males at 5 and 10 mg/kg bw/day. These changes were indicative of liver damage and increased in severity with dose.

There were reductions in urea and creatinine in males at 10 mg/kg bw/day, reflecting poor clinical conditions or generalized toxicity.

Ophthalmoscopy did not show abnormalities.

Urinalysis: some color changes were seen. At top dose, urobilinogen and bilirubin was increased in both sexes and to a lesser extent at 5 mg/kg, bw/d. High levels of protein and traces of glucose were seen in some animals at 10 mg/kg bw/day. Some animals at top dose had blood in urine. This was confirmed by cytology. These changes are indicative of jaundice.

Organ weight: in the absence of pathological changes, the increased kidney weight in males is considered to be of no toxicological significance.

Macroscopic findings: dogs killed inter-currently were thin and showed marked yellow discoloration of tissues. Livers were pale with accentuated lobular pattern. The female dog had a distended gall bladder. Similar findings were recorded in animals killed at scheduled termination. In one male dosed at 10 mg/kg bw/d, thymus gland size was reduced.

Microscopic changes: the 2 dogs killed inter-currently had marked hepatitis with hepatocyte necrosis and degeneration, inflammatory cell infiltration, increased pigmentation in hepatocytes and Kupffer cells, and biliary proliferation. The liver change of biliary proliferation with inflammatory cell infiltration, seen in 2 dogs at 5 mg/kg bw/d and in 1 female at 1 mg/kg bw/d was considered a less severe form of the hepatitis present at 5 and 10 mg/kg bw/d. In animals killed at scheduled termination, treatment-related findings were similar to those present in inter-current dogs. At the different dose levels, some dogs showed bile duct proliferation and portal inflammatory cell infiltration with/without minimal macrophage pigmentation.

The minimal-slight increased mitosis of the urinary bladder epithelial cells represents an increase turnover of cells since there was no evidence of hyperplasia and is considered to be related to treatment with metam sodium. At 10 mg/kg bw, a single occurrence of thymus atrophy, testes and prostate gland immaturity in appearance were considered to be non-specific effect of severe toxicity of metam sodium.

Table B.6.3.2.2-1a: 90-day gavage study in dogs with metam sodium.

Endpoints/dose	0		1		5		10 mg/kg bw/d	
	Number affected animals/number examined							
Sex	M	F	M	F	M	F	M	F
Mortality							1 killed wk 12	1 killed wk 11
Clinical signs								
Salivation from wk 3							¾	4/4
Food regurgitation					¾	¾	2/4	¼
Jaundice							2/4	¼
Body weight: week 10-14							↓5-14 % *	
Hematology week 13								
Kaolin-cephalin time							↑18%*	
Protrombin time							↑29%*	↑12%
MCV					↑3%	↑3%	↑7.6% *	↑6% *
MCH							↑4% *	↑2.6%
MCHC							↓3% *	↓4% *
Lymphocyte count			↑35% *		↑59% *		↑31%	
Monocyte count						↑158%*	↑119%*	↑311%*
Blood chemistry: week 13								
ALT				↑371%*	↑1400%*	↑596%*	↑2395%*	↑794%*
AST					↑141%*		↑115.3*	↑108%*
ALP					↑238%*		↑660%*	↑317%*
Creatine kinase							↑95%*	
Bilirubin total							↑2500%*	
GGT							↑487%*	↑400%*
Urea							↓37 % *	
Glucose							↓16 % *	
Cholesterol							↓29 % *	
Sodium								↑2.2%*
Creatinine							↓25 % *	
Chloride								↑2.7%*
Organ weight relative:								
Kidneys							↑25 % *	
Histopathology:								
Liver: hepatitis total	0	0	0	0	3/4	2/4	4/4	4/4
Biliary inflammatory cell infiltration	0	0	0	¼ slight	¼ minimal	¼ minimal	0	0

Endpoints/dose	0		1		5		10 mg/kg bw/d	
	Number affected animals/number examined							
Sex	M	F	M	F	M	F	M	F
Urinary bladder:								
Increased mitosis	0	0	0	0	1/4	0	3/3	2/3

*Statistically significantly different from control.

Conclusion:

Oral dosing with metam sodium caused treatment related hepatitis in male and female dogs dosed with 5 or 10 mg/kg bw/d, which resulted in the early termination of two dogs in the top dose group due to adverse clinical signs. The onset of this treatment-related hepatitis showed a clear dose and time relationship. An early indication of this effect was marked increase in ALT followed by increases in ALP and AST. The progressive inappetence and weight loss occurred later and coincided with increases in plasma GGT activity, total bilirubin and jaundice. Marked hepatitis was seen microscopically. There was a variety of changes, which were considered secondary to the hepatotoxicity or indicative of generalized toxicity or poor clinical conditions.

Already Peer Reviewed, EFSA 2008

NOAEL = 1 mg/kg bw/d,

LOAEL = 5 mg/kg b.w./d, taking into account the increased urinary bladder cell mitosis, liver hepatitis and clinical signs of toxicity.

The NOAEL of this study was reviewed by EFSA

a) It was questioned whether the lowest dose of Metam sodium in the 90d study was a LOAEL on the base of the ALT activity rise at 1 mkd. The average value (see table B.6.3.2.2-2) was significantly ($p \leq 0.05$) increased 8x, and 4.5x, as compared with pre-dose values and study control values, respectively. While it was comprised in the individual HCD, it was 1.7x above the range of means in these HCD.

Table B.6.3.2.2-2: Mean Alanine-Aminotransferase (ALT, IU/L) values in ♂ and ♀ Beagle Dogs (all doses) (90d capsule administration study, Brammer, 1992)

Sex	Study week	Dosage level (mg/kg bw/d)			
		0	1	5	10
♂	Predosing	17.5 ± 3.5	15.8 ± 3.8	19.3 ± 3.3	14.8 ± 4.8
	2	19.3 ± 2.2	17.5 ± 1.7	16.5 ± 3.9	13.8 ± 1.5
	4	22.8 ± 4.2	22.0 ± 2.9	21.0 ± 2.8	35.0 ± 33.5
	6	22.0 ± 5.3	21.0 ± 8.0	24.5 ± 5.4	139.3 ± 97.5**
	8	22.8 ± 2.6	18.8 ± 5.9	36.0 ± 20.5	184.3 ± 128.2*
	10	24.5 ± 3.8	25.8 ± 9.5	88.8 ± 97.2	916.8 ± 1157.3**
	12	27.0 ± 10.2	24.5 ± 7.8	378.8 ± 410.0**	423.5 ± 173.8**
	13	28.5 ± 3.8	29.0 ± 8.5	429.3 ± 451.6**	711.3 ± 283.2**
♀	Predosing	21.0 ± 2.0	17.5 ± 6.9	20.0 ± 5.6	16.5 ± 3.9
	2	23.0 ± 2.4	20.8 ± 11.3	15.3 ± 4.1**	14.8 ± 1.7*
	4	23.8 ± 5.9	25.5 ± 12.2	19.5 ± 4.5	35.8 ± 25.3
	6	32.0 ± 15.2	22.8 ± 12.2	19.3 ± 7.0	38.3 ± 35.9
	8	36.8 ± 17.3	24.0 ± 12.9	24.5 ± 13.5	92.5 ± 103.1
	10	28.0 ± 2.9	26.5 ± 12.3	49.8 ± 37.2	120.0 ± 97.8*
	12	23.5 ± 1.7	29.5 ± 10.9	161.0 ± 217.8*	240.0 ± 135.3**
	13	29.8 ± 9.0	134.5 ± 213.2*	207.5 ± 201.7**	266.7 ± 28.4**

Table B.6.3.2.2-3: Historical Control Data values (ALT, IU/L) in Beagle Dogs

(range of means or of individual values)

week	Range of means	
	♂	♀
Predosing	15.5 – 23.5	15.8 – 42.5
4	17.8 – 27.5	16.3 – 48.8
8	15.8 – 26	17.5 – 60.3
13	18.5 – 32.5	19.8 – 78.5
Range of individual values		
	♂	♀
Predosing	11 – 30	12 – 107
4	11 – 37	11 – 121
8	7 – 49	11 – 145
13	12 – 65	15 – 162

Inspection of the individual data in the ♀ revealed that the rise was due to one ♀ (#14), which showed a moderate time-dependent increase of ALT activity, but precipitously increasing from wk12 to wk13 (please refer to B.6.3.2.2-3).

Table B.6.3.2.2-3:

Individual values ALT activity (IU/L) of the 4♀ dosed at 1 mg/kg b.w./d during 13 weeks

week	♀ #13	♀ #14	♀ #15	♀ #16	m	±	s.d.
predosing	13	27	12	18	17.5	±	6.9
2	18	37	11	17	20.8	±	11.3
4	20	43	15	24	25.5	±	12.2
6	16	41	16	18	22.8	±	12.2
8	19	43	14	20	24.0	±	12.9
10	24	44	15	23	26.5	±	12.3
12	30	44	18	26	29.5	±	10.9
13	38	454	18	28	134.5*	±	213.2

Although only one ♀ was involved, the effect could be regarded adverse, since the liver is known to be the target organ, and in the view of similar increases at the higher doses.

However, as a 1 yr-dog study was present, from which the most relevant long-term NOAEL could be derived, a downwards revision of the 90d-NOAEL would not change the conclusions on the proposed reference doses.

Discussion:

It was discussed whether the increase of ALT (starting around 13th week) in one female in the 1 mg/kg bw/day dose group should be regarded as an outlier, or as the LOAEL. The finding appeared to be dose-related (effect also seen in one year study), but doubts were expressed whether it is really a critical finding. It was difficult to determine its significance as no information on liver weights in the study or histopathology data on the liver at 1 mg/kg bw/day were available. Although only one dog was affected at 1 mg/kg bw/day, the number of animals treated was small.

As a weight-of-evidence, it was considered a NOAEL. It was noted the NOAEL is lower for the one year dog study.

Remark:

The severe hepatic effects in the dog 90d study were discussed for the classification and labelling purpose.

At the top-dose of 10 mkd, 2/8 animals were terminated because of severe hepatic dysfunction (liver enzymes, hepatitis). Total hepatitis was observed histopathologically in 5/8 and 8/8 animals at 5 and 10 mkd respectively.

Notifier argued that on the basis of reversibility (on only 2 top-dose animals at 8 wks post-treatment), the effects were insufficient to trigger classification. RMS on the contrary is of the opinion that the need for classification Xn, R48/22 was justified. If doses between 10 and 50 mkd (limit-dose for classification) had been tested*, it is obvious that more fatalities would have occurred, and the argument of reversibility in so few animals at 10 mkd cannot be valid to leave the a.s. unclassified for subchronic toxicity hazards. However, allometric scaling considerations, highlighted by the notifier to indicate that effects at 10 mkd in dogs would not justify classification (as cut-off values would only be defined for 90d-rat studies), are not further supported in the EC

C&L discussions. As for other compounds, RMS considered that serious and irreversible effects in a subchronic dog study at <50 mg/kg bw/d warrant classification.

Further note of RMS: In a previous* 9 wk RF study with 1 animal/sex/dose (study not submitted, but from DPR review), it was reported that “*severe toxicity and death occurred in animals first dosed at 15 and 25 mkd*”, further supporting the findings.

RMS noted that at the top-dose of 10 mkd, 2/8 animals were terminated because of severe hepatic dysfunction (liver enzymes, hepatitis). Total hepatitis was observed histopathologically in 5/8 and 8/8 animals at 5 and 10 mkd respectively.

It was noted the classification assessment criteria does not mention other species (e.g. dogs) but only rats. However, it was considered that findings in other species may be taken into account if appropriate.

Conclusion: R48/22 was proposed on the basis of severe hepatotoxicity in dogs at 10 mg/kg bw/day.

GLP: yes, but no attest of competent authority.

Guidelines: not fully in agreement with Dir 87/302 or OECD 409 (1998-1981).

Deviation from official protocol: doses were too high.

Material and Methods:

4-beagle dogs/sex/dose were dosed with 0, 1, 5 or 10 mg/kg bw/d metam sodium in gelatin capsule (ref n° BAS 005 00N with CTL ref n° Y06930/008 at 43.15%) for a period of at least 90 days.

Statistical analysis: analysis of covariance for bodyweights on initial bw. Hematology, blood and urine chemistry, except ALT, AST and AP were considered by analysis of variance. ALP, ALT and AST were considered by analysis of covariance on pre-experimental values. Organ weight was analyzed by variance. Differences from controls were tested statistically by comparing each treatment group least square mean with the control group using a two-sided Student's test based on the error mean square in the analysis.

The study is accepted.

- Assessment of recovery in dogs (Brammer, 1993)

Findings:

Dosing ceased after 12 weeks for the female dog and after 13 weeks for the male. Recovery was then monitored for 8 weeks after the cessation of dosing, prior to termination.

The female dog took longer to eat during the last week of dosing and left a small food residue on 2 occasions during week 12. The male dog showed no clinical signs. There was no effect on body weight.

Individual plasma ALP and ALT activities were changed from week 6 in the female and from week 9 in the male and sustained elevations in plasma ALT (of over 200IU/L) were observed in weeks 10-12 and weeks 11-13 for the female and male, respectively. Following cessation of dosing, a further small increase in ALT was observed in both dogs, followed by gradual decline in the enzyme activities, with plasma ALT recovering more slowly than plasma ALP. By the end of the recovery phase (8 weeks after cessation of dosing), the pre-study ALT values had been virtually regained.

There were no macroscopic abnormalities in either dog and liver weights were normal. Microscopically, the only histological change present in the liver was a minimal or slight increase in the number of pigmented macrophages/Kupffer cells.

Conclusion:

Considerable reversal of both macroscopic and microscopic changes in the liver had taken place between cessation of treatment with metam sodium and termination of the study. The liver enzyme changes were reversible with full recovery eight weeks after cessation of dosing.

Material and Methods:

One beagle dogs/sex was dosed with 10 mg/kg bw/d metam sodium in gelatin capsule (ref n° BAS 005 00N with CTL ref n° Y06930/008 at 43.15%) for a period of at least 90 days.

The study is accepted.

B.6.3.2.3a Oral 1 year toxicity (dog) (Annex IIA 5.3.2)

- Metam sodium: 1 year gavage study in dogs at 0.05, 0.1 or 1 mg/kg bw/d (Brammer, 1994)

Findings:

There were no deaths.

The presence of salivation at dosing is considered a response associated with dosing and/or feeding and as such, is of no toxicological significance. A similar response has been observed in control dogs on other capsule dosing studies.

There were no ophthalmoscopic abnormalities in animals.

There were no toxicologically significant effects on body weight, food consumption, and clinical conditions or on the incidence of gastro-intestinal abnormalities.

Hematology: an increase in coagulation time observed at top dose could suggest toxicity.

Clinical chemistry: group means ALT were generally increased in the female 1mg/kg bw from week 8. One female showed consistently raised values in ALT from week 26 and an increase in AST, indicative of hepatotoxicity. A male dog in this group also showed a similar profile of slightly raised plasma ALT in weeks 32-45 with a sharper increase in week 52. Slight increases in plasma ALT were seen in individuals in other treatment groups; for one male (0.1 mg/kg) and one male (0.05mg/kg) these increases were accompanied by elevations in AST and creatine kinase. The plasma alkaline phosphatase activities of one female at 1 mg/kg were higher than controls in the second half of the study.

Increase in mean plasma ALP and a reduction in plasma triglycerides may also indicate a more generalized hepatotoxicity.

Histopathology: treatment-related findings were confined to the liver of two females at 1 mg/kg bw/d and comprised a slight increase in hepatocyte and macrophage /Kupffer cell pigmentation, slight mononuclear cell infiltration and slight telangiectasis affecting one female dog.

Table B.6.3.2.3-1a: 1-year gavage study in dogs with metam- results of week 52-53.

Table 20.2.2.1a. 1-year gavage study in dogs with melamin. Results of week 52-53.								
Endpoints/dose	0		0.05		0.1		1 mg/kg bw/d	
	Number affected animals/number examined							
Sex	M	F	M	F	M	F	M	F
Clinical signs:								
salivation					1/4	1/4		1/4
Body weight						↓8%	↓4 %	↓8 %
Food consumption			No significant food residues					
Hematology:								
APTTs							↑3.6%	↑9.8%*
Blood chemistry:								
Plasma sodium								↑1.4%*
Plasma chloride							↑2.8%*	↑2.1%*
ALP							↑42%*	↑46%*
ALT								↑279%
Triglycerides								↓34%*
Microscopy:								
Liver:								
hepatocyte pigmentation	0/4	1/4	0/4	0/4	0/4	0/4	0/4	2/4
macrophage/Kupffer cell pigmentation	1/4	1/4	0/4	0/4	0/4	0/4	0/4	2/4
Slight telangiectasis	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4

*Statistically significantly different from control.

Conclusion:

The laboratory proposed to set the NOAEL at 0.1 mg/kg bw/d. RMS agrees with this proposal, not taking into account the effects seen in female body weight, which are not statistically significant, and there is no clear dose effect relationship.

NOAEL = 0.1 mg/kg bw/d based on the slight effects seen at 1 mg/kg bw/d suggesting hepatotoxicity, blood chemistry effects, and associated histopathological findings.

RMS initially did not support the proposal of the company to set the NOAEL at 1 mg/kg bw/d. The argumentation of the company is presented below.

- Findings made on haematological and clinical-chemical examination were not dose-related or not consistently seen during the study period or due to individual animals in both control and treated groups
- Most of the changes observed in haematology and clinical chemistry were covered by the range of historical background data and were therefore not regarded to represent toxicologically adverse effects
- Effects on liver enzymes (ALT + ALP) were demonstrated to be reversible, to be within the historical control data range and were not correlated to histopathological findings in the liver other than those observable for control animals;
- Macroscopic and microscopic examination revealed no treatment-related adverse effect in any group of treated male and female dogs and the type, incidence and severity of macroscopic/microscopic findings in organs of treated groups were comparable to controls or findings were slight in degree and observable in one individual dog only; in addition, findings in the liver of one high dose female are of minimal to slight severity only and therefore not considered to represent a toxicologically adverse effect
- Only an NOEL derived in the study which reflects conclusion of the study director and no differentiation made between NOEL and NOAEL.

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RMS: The 3× increased ALT activity at 1 mkd in the 1 yr-dog study replicated the finding observed in a dose-dependent way in the 90d-dog study at ≥1 mkd, with concomitant hepatic necrosis from 5 mkd onwards (the liver being the target organ throughout all oral toxicity studies). Therefore, the overall assessment indicates that the enzymatic changes at 1 mkd and higher could be regarded adverse, and taken into account for the determination of the NOAEL.

Table B.6.3.2.3-2

Mean Alanine-Aminotransferase (ALT, IU/L) values in ♀ Beagle Dogs (control and top-dose) (52 weeks capsule administration study, Brammer, 1994)

Study week	Dosage level (mg/kg bw/d)	
	0	1
predosing (-4)	19.0 ± 3.5	20.3 ± 1.5
4	23.8 ± 5.6	24.8 ± 3.1
8	22.5 ± 5.4	30.5 ± 6.0
13	29.5 ± 13.8	38.0 ± 13.6
19	29.0 ± 11.0	40.8 ± 18.1
26	29.3 ± 5.5	103.3 ± 132.8
32	25.3 ± 4.3	102.8 ± 150.3
39	33.0 ± 3.4	90.0 ± 110.5
45	36.0 ± 9.4	128.5 ± 181.4
52	42.0 ± 20.3	125.0 ± 183.5

Inspection of the individual data in the ♀ revealed that the rise was due to one ♀ (#229), which showed a sudden increase of ALT activity, from wk19 to termination (please refer to B.6.3.2.3-3). Like in the 90d study, one ♀ dog showed high ALT values at 1 mkd, which was taken into consideration for the setting of the LOAEL.

Table B.6.3.2.3-3:

Individual values ALT activity (IU/L) of the 4♀ dosed at 1 mg/kg b.w./d during 52 weeks

week	♀ #229	♀ #230	♀ #231	♀ #232	m	±	s.d.
predosing (-1)	22	19	21	19	20.3	±	1.5
4	25	23	29	22	24.8	±	3.1
8	33	23	37	29	30.5	±	6.0
13	46	27	53	26	38.0	±	13.6
19	64	22	45	32	40.8	±	18.1
26	302	31	49	31	103.3	±	132.8
32	328	20	31	32	102.8	±	150.3
39	255	26	30	49	90.0	±	110.5
45	400	28	30	56	128.5	±	181.4
52	400	31	25	44	125.0	±	183.5

In addition, the NOAEL was initially established at 0.1 mkd also on the basis of histopathological findings, including hepatocyte and Kupffer-cell pigmentation at the next dose, pointing towards hepatotoxicity.

Discussion:

Based upon the observed adverse findings, which were much more clear than in the 90d study, the NOAEL was confirmed at 0.1 mg/kg b.w./d (and the LOAEL = 1 mg/kg b.w./d).

GLP: yes, but no attest of competent authority.

Guidelines: not fully in agreement with Dir 87/302/EEC Annex V or OECD 452 (1981).

Deviation from official protocol: ornithine decarboxylase was not measured; ovaries were not weighed and selected doses are too low.

Material and Methods: Four beagle dogs/sex/dose were dosed by gavage with metam sodium (batch reference n° BAS/005/00N 90-2; CTL n° Y06930/008; 525.54 g/L (43.148% w/w)) at 0, 0.05, 0.1 or 1 mg/kg bw/d for a period of at least 1 year and were then killed. Gelatin capsules were prepared daily by adding metam sodium to purified water. Control animals received vehicle only. The achieved dose levels were within 10% of the nominal concentrations and the chemical stability was satisfactory.

Statistical analysis: analysis of covariance for males and females body weight, hematology, clinical chemistry. Urinalysis and plasma GGT were considered by analysis of variance. Organ weight was considered by analysis of variance and covariance on final body weight.

Differences from control were tested by comparing each group least square mean with control group least square mean using a 2-sided Student's t-test.
The study is accepted.

B.6.3.3.1a 21-day inhalation toxicity (rat) (Annex IIA 5.3.3)

- 21 day inhalation toxicity of metam fluid preparation of methylcarbomodithioic acid sodium salt-called for short "metam fluid" in rats (Leuschner,1979)

Findings and conclusions: during the treatment with metam fluid by inhalation at 0.51 mg/L, no influence of the test compound was seen. At 1.54 mg/L, the body weight gain was reduced for both sexes during the whole study and hemoglobin was increased as well as leucocytes in females. Pulmonary lesions, mostly alveolar emphysema were observed in 17 rats.

The highest dose of 4.53 mg/L is within the lethal range. In this group, the body weight gain was inhibited parallel to food intake. Hemoglobin was increased and RBCs and platelet counts were increased as well as hematocrit. Reticulocytes and leucocytes were reduced. 1/10 female rats died showing ataxia, lack of appetite, haemochromodacryorrhea and unkempt coat. Pulmonary lesions were reported as well as thymus atrophy, changes in urinary bladder, inflammatory nasal cavity, inhibited spermatogenesis and prostate atrophy. The NOAEL can be expected to be = 0.51 mg/L breathing air.

GLP: no

Guidelines: not fully in agreement with Dir 87/302/EEC or OECD guideline 413(1984).

Deviation from official protocol: dosage duration and administration is not clearly reported (exposure system, measures of particle diameter etc...).

Material and Methods: 25 Sprague-Dawley rats/sex/dose were exposed to metam fluid (lot n° 42.4% metam fluid at 510 g/L) via inhalation for 4 hours/day, at concentrations of 0.51, 1.54 or 4.53 mg/L for 21 exposure days in an open system.

Actual concentration in the exposure chamber is on the average/day: 0.51 ±0.03 mg/L breathing air; 1.54±0.06 mg/L breathing air and 4.53±0.19 mg/L breathing air.

The study is not accepted.

B.6.3.3.2a 65-day inhalation toxicity (rat) (Annex IIA 5.3.3)

- Metam sodium: subchronic inhalation study with Vapam technical in rats (Knapp, 1983)

Findings:

Mortality: One high dose male died and microscopic examination revealed numerous uroliths in urinary bladder and kidney and death was attributed to renal failure.

Body weight of animals at top dose was significantly decreased (males: 6-11%; females: 10-14%) starting from week 1 up to the end of the study. Body weight gain compared to controls was reduced at all dose levels.

Food consumption decreased significantly at 32 and 160 mg/m³ suggesting a slight treatment effect.

Clinical signs: chromorhinorrhea incidence was increased and median time of onset was decreased at 32 and 160 mg/m³. Associated with the chromorhinorrhea was an increased incidence of wet coat and dry stains. Seminal plugs were frequently observed in the waste pans of animals at 32 and 160 mg/m³ following the daily exposure. The mean number of seminal plugs was increased at top dose and this could suggest mild stress.

There were no treatment-related ophthalmic effects.

Clinical chemistry: in females, at different dose levels, decreases in total serum protein, albumin and/ or α₂globulin were seen and a significant increase in α₁ globulin was seen at top dose; the biological significance of these effects is uncertain. Albumin decrease suggests impaired liver function. ALP was increased in females at 32 and 160 mg/m³.

Organ weight: the increase in relative liver weight in females at top dose could be related to hepatic dysfunction as suggested by decreased albumin concentration and increased ALP.

Necropsy findings: focal red and/or brown discolorations on the glandular stomach of animals at top dose are compound related.

Organ weight: relative weight of different organs was significantly increased at top dose. The relative adrenal weights were increased significantly in females exposed at top dose and this is considered as evidence of stress.

Histopathology related to exposure:

- Nasal passage: Histopathology revealed signs of irritation stress in the nasal passages (lymphocytic rhinitis and mucigenic epithelial hyperplasia), achieving statistical significance at the top two doses. At no dose did the severity of these lesions rise above “very slight.”

- Stomach: various stages of erosive gastritis were found in the glandular mucosa at top dose. The incidence and severity was slightly higher in female than male rats. According to the company, the effect on stomach is considered stress induced rather than resulting from direct irritation to the gastric mucosa.

- Liver lymphogranulomatous hepatitis (very slight) was seen in males at top dose. A re-examination of liver sections was performed and reported below by Duffel, 1992.

- Pulmonary histiocytosis was reported at top dose.

The other microscopic observations were not considered related to exposure to Vapam technical.

Table B.6.3.3.1-1a: 13-week inhalation study with Vapam- results of week 13.

Endpoints/dose	0		6.5		45		160 mg/m ³	
Corrected dose mg/kg bw/d								
Metam sodium	0		1.75		12.1		42.6	
MITC	0		0.189		0.59		1.54	
	Number affected animals/number examined							
Sex	M	F	M	F	M	F	M	F
Mortality		1, wk 6					1/18 week 9	
Body weight							↓6%	↓8%*
Bw gain			↓16%	↓17%	↓42%	↓17%	↓67%	↓32%
Food consumption			↓3%		↓8%*		↓10%*	
Hematology:	No compound related effect							
Blood chemistry:								
LDH (week 6)				↓36%*		↓50%*		↓61%*
Total protein				↓10%*		↓10%*		↓18%*
Albumin						↓9%*	↓13%*	↓26%*
Total globulin								↓13%*
Globulin α1					↑50%*		↑50%*	↑40%*
α2				↓50%*				↓50%*
Glucose								↓29%*
ALP						↑100%*		↑118%*
Necropsy findings:								
Lung colored foci	1/18	0/17	3/18	1/18	1/18	0/18	3/17	3/18
Stomach colored foci/streaks	1/18	0/17	0/18	0/18	0/18	0/18	4/17	14/18
Skin brown staining				1/18		4/18		4/18
Relative organ weight								
liver								↑14%*
Brain								↑11%*
Heart								↑9%*
Kidneys							↑6%*	↑14%*
Adrenals								↑30%*
Lungs							↑13%*	↑41%*
Gonads								↑31%*
Histopathology:								
Lung: histiocytosis	0	0	0	0	1/18	0	3/17	2/18
Nasal passage: lymphocytic rhinitis	1/18	1/17	4/18	3/18	6/18*	4/18	7/17*	5/18
Hyperplasia of mucigenic epithelium very slight	5/18	1/17	1/18	6/18	9/18	10/18*	13/17*	11/18*
Mucigenic cysts	0/18	0/17	0/18	1/18	0/18	1/18	0/17	2/18

Endpoints/dose	0		6.5		45		160 mg/m ³	
Liver:								
Lymphogranulomatous hepatitis very slight	0	0	1/18	0/18	2/18	2/18	12/17	0/18
Necrotic hepatitis	1/18	1/17	2/18	0	0	0	0	0
Biliary hyperplasia very slight	2/18	3/17	2/18	1/18	1/18	1/18	3/17	3/18
Stomach Ecstasy, glandular duct	0	0	0	2/18	0	1/18	0	4/18
Erosive gastritis	1/18	0/17	0	0	0	0	9/17*	13/18*
Ulcerative gastritis	0	0	0	0	0	0	0/17	2/18
Kidney pyelitis	0	0	0	0	0	0	0	2/18
Urinary tract hyperplasia transitional epithelium	0	0	0	0	0	0	0	1/18
Metaplasia	0	0	0	0	0	0	0	1/18

*Statistically significantly different from control.

Conclusion:

RMS considers that the NOAEL must be set at 6.5 mg/m³ = 1.75 mg/kg bw/d for metam sodium: Based on food consumption decrease, serum chemical changes, some of which may indicate liver damage and nasal stress revealed by histopathology.

Pulmonary histiocytosis and gastric erosions of the stomach were seen in male and female rats exposed to 160 mg/m³.

The company proposes to set the NOAEL at 45 mg/m³.

The NOAEL deduction of this study done by the notifier was in accordance with the evaluation in the study report and the NOAEL was, thus, derived at the mid dose level. The notifier is of the opinion that changes in blood parameters are not of any biological and toxicological relevance since re-examination of the liver sections did not reveal a treatment-related effect on livers and changes on these blood parameters are thus, not indicative for hepatotoxicity due to the absence of a histological correlation in the liver and also in other organs. Furthermore, no treatment- and/or dose-related effect were evident on absolute and relative organ weights at the low and mid dose level. Reductions in body weight gain at the mid dose level of both sexes were not accompanied by corresponding reductions in absolute body weights which were very comparable to controls at the low and mid dose level. As can be seen from the body weight tables, the body weight gains determined at the different time points were very comparable to controls at the low and mid dose levels. However, absolute body weights were about 15 % higher in animals assigned to treatment groups at study initiation which explains the retardations in overall body weight development at study termination.

The effects observed in the respiratory tract are considered to be of local nature due to the irritating properties of metam.

Based on the observations and findings made in this study, the overall conclusion presented in the study report is considered to appropriately assess the NOAEL for systemic effects which is derived at the mid dose level of 45 mg/m³.

GLP: yes, but no attest of competent authority.

Guidelines: not fully in agreement with Dir 87/302/EEC or OECD guideline 413(1981).

Deviation from official protocol: blood analysis not performed in all animals. The study is performed with a formulation.

Material and Methods: 18 Sprague-Dawley rats/sex/dose were exposed to aerosolized Vapam technical (lot n° EHC 0355-21; containing 32.7% metam sodium) via inhalation for 6 hours/day, 5 days/week at concentrations of 0, 6.5, 32 and 160 mg/m³ for 65 exposure days. Blood samples were obtained from 6 rats/sex/dose after 6 weeks of exposure and from abdominal aorta of 6 rats at study termination.

Inhalation chambers were of 1 m³ and proposed concentrations were generated using a Collison compressed air nebulizers. Metam sodium decomposed gradually to MITC and H₂S, in a 1 to 1 ratio, upon exposure to humidity in the chamber atmosphere. Periodically the concentration of MITC was determined. Actual mean chamber concentrations after 65 days of exposure were 6.5, 45 or 160 mg/m³. These values, which were determined using measurements of sodium ion levels, were calculated to be equivalent to internal doses of 1.75, 12.1 or 42.6 mg/kg/day. Mean MITC concentrations, measured by infrared adsorption, were 0.7, 2.2 or 5.7 mg/m³ (0, 0.23, 0.74, and 1.90 ppm), equivalent to internal doses of 0.189, 0.59 or 1.54 mg/kg/day.

Statistics: body weight, hematology, blood chemistry, cholinesterase, and organ weights were compared between groups by one-way analysis of variance, Bartlett's test for homogeneity of variance and Dunnett's t test. Clinical observations were analyzed by Mann-Whitney sample rank test or Fishers exact probability test. Histopathology was analyzed by the Fishers exact probability test. The study is accepted.

Concentration of metam sodium in air (mg/m ³) and % of chamber atmosphere	MMAD	Mean concentration of MITC mg/m ³
6.5 mg/m ³ = 33% of chamber atmosphere	1.8 µm	0.7 = 0.23 ppm
32 = 74%	1.7	2.2 = 0.74 ppm
160 = 93%	2	5.7 = 1.9 ppm

- Metam-sodium: a CTL re-examination of liver sections from a sub-chronic inhalation study with vapam technical in rats (Duffell, 1992)

Findings:

In the previous reported study, the findings recorded here as mononuclear cell infiltration was termed lymphogranulomatous hepatitis. At the CTL, a mononuclear cell infiltration of the liver is recorded as part of the background pathology of the rat strains. Its severity is usually minimal to slight. It comprises focal or multiple deposits of lymphoid and macrophage cell types, in the portal areas and/or sinusoids, and is insignificant in terms of liver function and health of the animal. In strict semantics an infiltration of lymphoid cells and macrophages may be termed lymphogranulomatous, and as these are inflammatory cells the suffix "itis" is added to the organ so infiltrated. These infiltrating mononuclear cells probably represent the liver's reaction to a low level of antigenic and infectious challenge, although the pathogenesis of the lesion is not established. It is a background lesion occurring to a minimal extent and does not justify a description of lymphogranulomatous hepatitis, which suggests a far more significant lesion.

On re-examination of the liver sections there was little difference between the groups in the incidence of this lesion, by whatever term it is designated. There is a difference between the original pathologist's report and this one. It is likely that the original pathologist had a slightly higher threshold where he started to record the finding. In all those cases where he noted the finding there is agreement with the grading as minimal (table B. 6.3.3.1-2).

Table B.6.3.3.1-2a: 13-week inhalation study with metam- re-examination of liver slides.

	Control		160 mg/m ³	
	M	F	M	F
N° examined	18	18	18	18
Necrotic hepatitis focal minimal	2	1	0	1
Biliary hyperplasia minimal	1	1	0	0
Hepatocyte vacuolation focal slight	0	2	0	0
Mononuclear cell infiltration minimal	16	13	16	14
Capsulitis	1	1	0	0

Conclusion: the lesion termed lymphogranulomatous hepatitis is now designed mononuclear cell infiltration of the liver. There is no difference in the incidence of this lesion between control and high dose rats.

GLP status: procedures and report not subject to QA audit.

Aim of the study: the histological liver sections from rats in the control and top dose groups were re-examined to determine the nature and incidence of the lesions noted as lymphogranulomatous hepatitis in the study when originally reported by Stauffer.

Methods: the haematoxylin and eosin stained slides of the liver of all animals in the control and high dose groups were examined microscopically. The study is accepted.

B.6.3.3.3a Percutaneous 21-day toxicity (rat) (Annex IIA 5.3.3)

3-weeks toxicity of metam fluid at 31.25, 62.5, 125 mg/kg bw/d in rabbits (Leuschner, 1979)

Findings:

Mortality: all rabbits survived the study.

Clinical signs: during the 21 days of local application, no clinical signs were seen.

Food and water consumption were within the normal range during exposure.

Body weight gain was not impaired.

Hematology: no compound-related effects were seen.

Clinical chemistry: values were within the normal ranges.

Urinalysis: no abnormalities recorded.

Ophthalmoscopy was normal.

Necropsy did not show pathological changes.

Organ weights were normal.

Histopathology did not demonstrate any further dose-related findings.

Skin reactions:

At the lowest dose, the skin was not injured.

At 62.5 mg/kg, locally slight erythema was seen from the 4th to 13th day onwards, sometimes accompanied by slight rhagades. Edema occurred also in rabbits and was hardly seen at the last application day.

At 125 mg/kg, locally slight erythema was seen from the 1st day onwards with formation of slight to moderate rhagades (fissures or crack in the skin). Edema occurred also in all rabbits during the first half of the application period and was intensified at the last application day. Skin injury was more severe.

Edema disappeared within 4 days, erythema within 10-11 days in the rabbits with follow-up period, after discontinuation of treatment.

Macroscopic autopsy findings:

No injury of the skin was seen at 31.25 mg/kg bw/d.

At 62.5 mg/kg bw/d, the treated skin showed slight erythema in several animals and edema only in one rabbit at time of dissection after three application weeks.

Dose-related more marked erythema and edema were observed in all rabbits locally at the dissection at the end of application phase.

Histology: slight epidermal-dermatitis in eight rabbits treated with 62.5 mg/kg bw/d at the end of exposure period. No injury was seen at the end of recovery period.

At 125 mg/kg bw/d, moderate to marked epidermal dermatitis was seen in all rabbits. After recovery period, the skin of three rabbits was free from pathological changes.

There were no definite differences between intact and scarified skin in any dose.

Table B.6.3.3.3-1a: skin reactions in rabbits following a 21-day dermal exposure to metam sodium.

Skin reactions day 0-21	0		31.25		62.5		125mg/kg bw/d	
	Number affected rabbits/n°exposed							
	M	F	M	F	M	F	M	F
Intact skin								
Edema								
Slight	0	0	0	0			1/3	1/3
moderate							2/3	2/3
Erythema								
Slight	0	0	0	0	2/3	1/3		
moderate							2/3	1/3
marked							1/3	2/3
Rhagades								
Slight	0	0	0	0			2/3	1/3
moderate							1/3	2/3
Abraded skin								
Edema	0	0	0	0				
Slight					1/3		1/3	1/3
moderate							1/3	2/3
Erythema	0	0	0	0				
Slight					2/3	2/3		
moderate					1/3		2/3	2/3
marked							1/3	1/3
Rhagades					1/3			

Skin reactions day 0-21	0		31.25		62.5		125mg/kg bw/d	
	Number affected rabbits/n°exposed							
	M	F	M	F	M	F	M	F
Slight							2/3	2/3
moderate							1/3	1/3
Epidermis dermatitis:								
Slight					8/10			
Moderate to marked							10/10	

Conclusions:

No systemic toxicity was observed. Local dermal effects were seen at 62.5 and 125 mg/kg bw/d. NOAEL for local effects = 31.25 mg/kg bw/d. NOAEL for systemic toxicity > 125 mg/kg bw/d.

GLP: not mandatory at that time.

Guidelines: not fully in agreement with Dir 92/69/EEC or 84/449 but in agreement with OECD 410 (1981).

Deviation from official protocol: The doses are too low and inefficient.

Material and Methods: 10 white Russian rabbits/sex/dose received metam sodium (BAS 00500-N 42.4%) by local administration to intact and abraded dorsal skin over 21 days at 31.25, 62.5 or 125 mg/kg bw/d for a 8 hour exposure/day. 0.8% aqueous hydroxypropyl-methyl-cellulose gel served as diluent and was used for the control group. The application site area was of 15 x 16 cm. the application site was not covered. After exposure, the backs of the rabbits were washed with warm water. Animals were maintained in cages where turning of whole body was not possible. Two animals/sex/group were observed for an additional 21 days. The study is accepted.

B.6.3.3.4a Percutaneous 90-day toxicity (rat) (Annex IIA 5.3.3)

No data required.

B.6.3.3.5a Additional studies published in the open literature:

- Immunotoxicological characteristics of sodium methyldithiocarbamate (Pruett et al., 1992)

Findings:

Major effects included decreased thymus weight at all time points; increased spleen weight after 10 or 14 days of exposure, increased bone marrow cellularity after 10 or 14 days of exposure, significant decreases in mature lymphocyte subpopulations which were greater in the thymus than in the spleen, relatively selective depletion of the major subpopulation of thymocytes (CD4⁺CD8⁺), and decreased body weight. Overall patterns of changes were consistent with the conclusion that metam rapidly depletes most CD4⁺CD8⁺ thymocytes, more slowly depletes a smaller number of mature lymphocytes in the thymus and spleen, and induces compensatory and/or detoxication mechanisms after 10-14 days of exposure. Subsequent experiments were done to assess selected immune function parameters. Metam at 50-300 mg/kg/day for 7 days caused substantial, dose-dependent suppression of NK cell activity. No suppression of antibody production *in vivo* or splenocyte responses to mitogens or allogeneic lymphocytes *in vitro* was detected. NK cell activity, thymus weight, and CD4⁺CD8⁺ thymocyte numbers were suppressed by dermal administration of SMD.

According to the RMS:

In this study, metam was given at high doses via gavage and bw decrement was 10% or greater suggesting that the study was performed at the MTD. Spleen relative weight was increased and thymus weight was decreased. Hemoglobin and hematocrit and lymphocyte count were decreased and leukocyte and neutrophil count was increased. Similar effects were reported in studies reported in the dossier but at much lower doses. These results does not change the conclusions reported in the DAR.

Material and methods:

Six Female C57 black/6 x C3H mice were used. Metam (Chem. service, lot 14-133, 90% and lot 35-73, 95%) was dissolved in water and was freshly prepared for each assay at 300, 225 or 150mg/kg bw/d. Initially, the compound was administered orally to female B6C3F1 mice at 300 mg/kg/day for 3, 5, 10, or 14 days. Body, liver, kidney, spleen, and thymus weights were measured. Selected hematological and bone marrow parameters were examined. Flow cytometric analysis was used to assess changes in lymphocyte subpopulations in the thymus and spleen, and production of antibody-forming cells *in vitro* was measured. In another experiment, metam was given orally for 7 days and immunology was done on day 8.

B.6.3b Short-term toxicity with Methylisothiocyanate (MITC) (Annex IIA 5.3)

B.6.3.1b Oral 28-day toxicity (Annex IIA 5.3.1)

- **Study on the 4-week Inhalation Toxicity of Methyl Isothiocyanate in rats at 5, 20 or 100 mg/m³ (Klimisch et al., 1987)**

Findings:

No deaths occurred during the study period.

Clinical signs: during exposure to 20 mg/m³, rats had eyelid closure, somnolence, and ruffled fur from the third day of exposure onwards. On the next morning before exposure, nothing abnormal was noted. At 100 mg/m³, these signs were seen at a greater extent during exposure. Reddish nasal discharge occurred in some animals as well as eye discharge, intensified head cleaning and difficulty in breathing, and stretched posture. Initially, the rats recovered until the next exposure. As the duration of the study increased, the signs were no longer reversible.

Body weight was depressed for the male rats at top dose starting from day 7 and for female rats at day 27.

Body weight change at top dose was significantly depressed for males throughout the exposure period. In females, a trend toward retardation was observed without reaching statistical significance.

Hematology: increase in leukocyte count at top dose in females, increase in neutrophilic polymorphonuclear granulocyte count in males at mid dose and in male and female rats at top dose could be related to the inflammatory process in the respiratory tract. Tromboplastin times were prolonged in male rats at top dose.

Clinical chemistry: urea and glucose values were significantly decreased in males and females of top dose, whereas a decrease in triglyceride and albumin concentrations was noted in males only. Increase in bilirubin was noted in male rats at top dose. A very slight increase in GPT and ALT were noted in females at top dose.

Organ weight: some absolute organ weights were modified: liver and kidney weight were decreased while lung weight was increased. It cannot be concluded if altered liver and kidney weight are compound-related.

Histopathology:

- Liver: no pathological changes were seen and it is difficult to assign the hyperbilirubinemia observed and the prolonged clotting time to a specific clinical picture since no pathological changes were detected in the liver and the red blood cell counts. It is therefore difficult to conclude if these findings are of a substance-induced nature, taking into account that the described effects occurred in one sex only.
- Lungs of all male rats and two female rats at top dose were found to have focal mucous-purulent bronchopneumonia with partly widened alveolar septa and activated alveolar cover cells. The epithelial cells in the bronchi and bronchiole were proliferated. The lungs of these two females and three males were emphysematous.
- Tracheal epithelial proliferation and single cell necrosis occurred in all male and female rats at top dose and one animal/sex was found to have a mucous-purulent inflammation.
- Catarrhal purulent rhinitis was observed in the nasal cavity of all animals at top dose and olfactory epithelium was atrophic. These findings occurred in a few animals of the control group. Focal squamous metaplasia in the area of the respiratory epithelium was found in three males and all females at top dose. These changes are considered compound related (table B.6.3.1-1b).

Comment from RMS: according to a scientific review panel on toxic air contaminants air resources board, State of California (2002) it was concluded that the olfactory epithelial atrophy S2, can be divided up as focal and non focal and an increase can be seen from 1/5, 2/5 between control and low dose, no further increase at the mid dose and going to zero at the high dose. If we are looking to atrophy non-focal, which is interpreted as a somewhat more serious level of atrophy, (focal islands of atrophy have spread and become indistinguishable from each other); it goes up with dose. This means that since the focal atrophy is going down and the non focal atrophy is going up, there is an increase in severity between the low dose and the mid dose, and that in fact, between the control and the low dose, there is an increase in focal atrophy, which becomes non focal at higher dose.

According to the open literature, degeneration resulting in olfactory epithelial atrophy is a common change after administration of an olfactory toxin. Regeneration of the olfactory epithelium results in squamous or respiratory metaplasia, which is presumably more resistant to the irritant effects of the substances, is a common response in rodent nasal passages following exposure to cytotoxic irritants (Gaskell, 1990, Morgan et al, 2000).

Table B.6.3.1-1b: 4-week inhalation study of MITC in rats.

Endpoints/dose	0		5 (1.7 ppm)		20 (6.8 ppm)		100 mg/m ³ (34 ppm)	
	M	F	M	F	M	F	M	F
Body weight							↓12-23% * from day 7-27	↓6% * day 27
Bw gain					↓18-8% from day 7-27		↓18-81% * from day 7-27	↓90-37% from day 7-27
Hematology:								
HematoQuick Test							↑*16%	
Leukocytes								↑*29%
Neutrophilic polymorphonuclear granulocytes					↑43%*	↑60%	↑195%*	↑176%*
Clinical chemistry								
Urea							↓*12%	↓*30%
Glucose							↓*16%	↓*14%
Triglycerides							↓*58%	
Albumin							↓*8%	
Bilirubin total							↑*92%	
GPT								↑*26%
ALP								↑*31%
Organ weight absolute								
Liver							↓*26%	
Kidneys							↓*16%	
Lungs							↑*71%	↑*70%
Histopathology:								
Liver:								
Fatty infiltration							2/5	0/5
Nasal cavity:								
Rhinitis catarrhal purulent								
Section 1	1	0		2	1	1	5	5
S2	1	0	0	1	0	0	5	5
S3	2	0	1	2	1	0	5	5
S4	1	0	0	1	1	0	5	4
Olfactory epithelium								
Atrophy focal								
Section 2	1	1	2	3	1	1	0	0
S3	0	0	1	0	2	0	0	0
S4	1	0	0	1	1	0	1	1
Atrophy								
Section 2	1	0	1	0	2	2	5	5
S3	2	0	1	2	1	1	5	5
S4	0	0	0	0	0	0	4	4
Respiratory epithelium								
Metaplasia section 1	0	0	0	0	0	0	3	5
S2	0	0	0	0	0	0	0	2
Trachea:								
Mucopurulent inflammation	0	0	0	0	0	0	1	1
Epithelial proliferation	0	0	0	0	0	0	5	5
Single cell necrosis	0	0	0	0	0	0	5	5
Lung:								
emphysema	0	0	0	0	0	0	3	2

Endpoints/dose	0		5 (1.7 ppm)		20 (6.8 ppm)		100 mg/m ³ (34 ppm)	
	M	F	M	F	M	F	M	F
Bronchopneumonia	0	0	0	0	0	0	5	2
Bone metaplasia	1	0	0	1	0	1	3	0
Epithelial proliferation	0	0	0	0	0	0	5	2

Statistically significant modification : *p<0.05; s1..s4: examined sections (stage of sectioning, possibly from rostrad –s1, to caudad –s4; note: no reporting of olfactory atrophy in s1 – reasons not mentioned in the study).

Notifier agreed with the proposal after reconsideration of the findings.

Conclusion:

Increase in lung weight, bronchopneumonia and epithelial proliferation in bronchi and bronchiole, proliferation in tracheal epithelial cells and inflammatory changes in the nasal cavity and atrophy of the olfactory epithelium as well as focal metaplasia of squamous epithelial cells in the area of respiratory epithelium were seen essentially at top dose of 100 mg/m³. The company proposed a NOAEL = 20 mg/m³ (5.76 mg/kg bw/d). However, RMS considers that due to the slight decrease in bw gain seen in males at 20 mg/m³, NOAEL systemic effects = 5 mg/m³ (= 1.35 mg/kg bw/d).

RMS agrees with the Californian scientific panel with regard to the interpretation of nasal atrophy. Therefore, RMS proposes to set the NOAEL local effects <5mg/m³ (= 1.35 mg/kg bw/d) based on the increased incidence of focal nasal atrophy reported at this dose level.

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Open point: 2.6 MSs to agree on the relevant NOAEL of the 28 day inhalation study with MITC (Klimisch et al 1987) at a meeting of experts [See reporting table 2(14)].

RMS : At 20 mg/m³, there is a slight decrease in body weight gain in males, suggesting that the NOAEL for systemic effects =5 mg/m³.

The decreased weight gains in the ♂ are reproduced in table B.6.3.1.1b.

Table B.6.3.1-1b: 4x5d inhalation toxicity of MITC in rats: body weight gain (g)

Dose (mg/m ³) ppm	0	5.1 1.7	19.9 6.8	100.0 34
	(g)	(g) %	(g) %	(g) %
d7	23.5	24.7 105	19.3 82	-19.2**
d14	42.6	45.6 107	37.8 89	-22.0**
d21	62.9	67.2 107	55.8 89	-28.2**
d27	79.7	83.6 105	72.7 91	-14.9**

RMS considers that focal atrophy is going down and the non-focal atrophy is going up, suggesting that there is an increase in severity between the low and mid dose and that in fact, between the control and low dose, there is an increase in focal atrophy, which becomes non focal at higher dose. The local effects NOAEL was considered <5 mg/m³.

GLP: only a statement of QAU

Guidelines: in agreement with Dir 92/69/EEC.

Material and Methods: Five Wistar rats/sex/dose were exposed 6 hour/ day by inhalation to MITC (batch n° 6205MK, 96.9%) as a vapor for 4 weeks each working day. The intended respiratory air concentrations were 5, 20 and 100 mg/m³, and the following values were obtained: 5.1, 19.9 and 100 mg/m³. 5rats/sex was kept as controls. Air samples were analyzed by GC and MITC quantities were calculated from the corresponding area integral value.

Statistical analysis was performed using the computer system of the department: clinical examination was performed using the ANOVA test and Dunnett's test; chemistry was compared using the t test and pathology evaluation was performed using a t test generalized by Williams. The study is accepted.

B.6.3.2.2b Oral 90-day toxicity (dog) (Annex IIA 5.3.2)**- Methyl isothiocyanate: 13 week oral toxicity study in Beagle Dogs (Final Report – Repeated administration for 13 weeks) (Harling et al, 1986)**Findings:

Mortality: there were no deaths.

Clinical signs: vomiting, usually within 2 hours of dose administration was occasionally reported in control, 0.04 and 0.4 mg/kg bw/d and occurred more frequently at 2 mg/kg bw/d.

Excessive salivation, associated with the dosing procedure was occasionally observed for 3 dogs receiving 2 mg/kg bw/d during the final 7 weeks of dosing.

Body weight: gain was reduced for most females at top dose. The rate of weight gain was increased for many animals due to the intake of 10 ml/kg/day corn oil as part of the dosing procedure.

Food consumption: no compound-related effects were seen in females. In males, slight not reaching statistically significance, dose related effect was noted.

Ophthalmoscopy: no treatment-related abnormalities were detected.

Haematology:

Mean activated partial prothrombin time for females from all treated groups during week 6, and males and females at 2 mg/kg/day during week 13 were significantly greater than the control value. The mean prothrombin time for males at top dose was also significantly increased during week 13.

Clinical chemistry: at week 6, there was an effect on serum proteins with significantly decreased total protein (at 2 mg/kg bw/d: ↓6% in males, ↓8% in females) and or total globulin concentrations reported for males (↓17%) and females (↓14%) at top dose during week 6 and during week 13 for females. The mean albumin concentrations for females in all treated groups were greater than control values during week 13 and could be related to dehydration. Electrophoresis during week 13 revealed decreases in all 4 globulin fractions (α1, α2, β, γ) for males and females.

Mean calcium levels for animals receiving 2 mg/kg were significantly decreased during week 6 and 13 of dosing.

Mean cholesterol concentrations were significantly lower than control in females but such a trend was apparent before dosing.

Urinalysis: no treatment-related effects were seen.

Macroscopy: post mortem: the mucosal surface of the gall bladder of most control and treated dogs was white and furry in appearance. The liver of many animals from control and treated groups were coarse in texture, with accentuation of the lobular pattern. Pale discoloration of the liver was sometimes also observed. These findings were partially attributed to the large volume of corn oil consumed by the animals. The severity of these findings increased with increasing dose level. According to the authors of the study this may be related to administration of the vehicle corn oil.

White catarrhal material adherent to the stomach mucosa was observed at 2 mg/kg bw/d. generalized pallor of the skeletal muscle was reported in 1 dog of the same group.

Organ weight: no compound-related effects.

Histopathology: periportal hepatocyte vacuolation and lipid deposition were found in the livers of control and treated dogs. However, both the incidence and severity were increased for animals receiving 0.4 and 2 mg/kg. A degree of thymus involution was observed at 0.4 mg and at 2 mg/kg (Table B.6.3.2.2-1b).

Table B.6.3.2.2-1b: 90-day gavage study in dogs with MITC results of week 13.

Endpoints/dose	0		0.04		0.4		2 mg/kg bw/d	
	Number affected animals/number examined							
Sex	M	F	M	F	M	F	M	F
Clinical signs:								
Vomiting	3/4	3/4	2/4	3/4	4/4	2/4	4/4	4/4
Body weight			↓5 %		↓0.5 %		↓4 %	↓12 %
Body weight change			↓7 %	↑30%	↓7 %	↑33%	↓17 %	↓54 %
Food consumption			↓3 %	↓11 %	↓5 %	↓3 %	↓8 %	↑2%
Hematology:								
Platelets							↑28%	

Endpoints/dose	0		0.04		0.4		2 mg/kg bw/d	
	Number affected animals/number examined							
Sex	M	F	M	F	M	F	M	F
PTs							↑8%*	
APTTs							↑20%*	↑17%*
Blood chemistry:								
Globulin								
Albumin				↑30%*		↑21%*		↑17%*
Total globulin								↓20 %*
α1							↓25 %*	↓12 %
α2							↑16%	↓17 %*
β							↓15 %	↓23 %*
γ				↓25 %*		↓25 %*	↓20%	↓25 %*
Ca ²⁺							↓7%*	
Cholesterol						↓26 %*	↓35%*	↓36 %*
Urinalysis:								
Volume			↓24 %	↓4 %	↑18%	↓33 %	↓13 %	↓30 %
PH	6.7	6.5	7.0	6.5	6.7	7.5*	6.3	6.8*
Macroscopic findings:								
Gall bladder-furry mucosa	7/8		8/8		8/8		8/8	
Liver:								
coarse texture	4/8		5/8		7/8		8/8	
pale discoloration	1/8		0/8		1/8		3/8	
accentuated lobular pattern minimal	2/8		2/8		4/8		3/8	
/moderate	2/8		2/8		3/8		5/8	
Histopathology:								
Thymus involution					1/4	1/4	2/4	1/4
Liver:								
hepatocyte vacuolation+ lipid depots:								
Moderate	0/4	0/4	0/4	0/4	0/4	1/4	3/4	2/4
Marked	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4

*Statistically significantly different from control.

Conclusion:

According to the RMS, the NOAEL should be set at 0.04 mg/kg bw/d taking into account, at the next dose level of 0.4 mg/kg bw/d of thymus involution, and liver periportal vacuolation and lipid deposition.

RMS does not support the proposal of the company to set the NOAEL at 0.4 mg/kg bw/d. The argumentation of the company is presented below.

- Clearly treatment-related and toxicologically relevant effects notable at 2.0 mg/kg bw/d (body weight/body weight development in females, prothrombin time in males/females, calcium in males and females, cholesterol in males and females, lung weight in males, macroscopic findings in stomach and skeletal muscle, moderate to marked liver hepatocyte vacuolation in males and females on microscopic examination)
- Changes in Albumin/Globulin at 0.04 mg/kg bw/d and 0.4 mg/kg bw/d not dose-related and/or not consistently seen at the specified observation time points and covered by range of historical background data taking into account age-dependencies
- No statistically significant changes in liver-specific parameters such as ALT/AST/ALP or Total Bilirubin in all groups of treated dogs
- Statistically significant decrease in testes weight at 0.4 mg/kg bw/d and 2.0 mg/kg bw/d and statistically significant increase in pancreas weight at 0.4 mg/kg bw/d and 2.0 mg/kg bw/d in females not regarded to be toxicologically relevant as no clear dose-response relationship notable and changes covered by range of historical background data taking into account age-dependencies

- *Macroscopic findings:* Coarse texture of liver also seen in control animals and regarded to be the consequence of large volumes of vehicle (corn oil) used
- *Microscopic findings:* Liver hepatocyte vacuolation in males and females only trace to minimal at 0.04 mg/kg bw/d and 0.4 mg/kg bw/d and not regarded to be an adverse and toxicologically relevant effect due to the absence of a clear dose-response relationship and no changes in liver serum enzymes
- *Conclusion derived in the study reflects conclusion of the study director and no differentiation made between NOEL and NOAEL.*

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Open point: 2.7 Relevance of thymus involution and liver vacuolation at 0.4 mg/kg bw/d in the study with MITC by Harling et al, 1986, to be discussed at a meeting of experts
[See reporting table 2(17)]

RMS: Initially, it was suggested to revise upwards the relevant 90d dog NOAEL for MITC. It is questionable to ignore the thymus effects at the mid-dose, as the finding was replicated at the top-dose, indicating a treatment-related effect. The same remark holds for the vacuolation and lipid depots, which occur in singularity at the mid-dose, but again observed in 5/8 animals at the top-dose. A trend toward coarse liver texture was also noted with incidences of 4/8, 5/8, 7/8 and 8/8 at 0/ 0.04/ 0.4 and 2 mkd. This trend indicates a possible implication of treatment at 0.4 mkd onwards. As several parameters were disturbed at the mid-dose, the NOAEL could be established at 0.04 mkd.

The abovementioned findings (for liver histopathology somewhat extended) are highlighted again in the next table.

Table B.6.3.2.2-1b: 90-day gavage study in dogs with MITC histopathology results of week 13
(Harling et al, 1986)

Endpoints/dose	0		0.04		0.4		2 mg/kg bw/d	
Sex	♂	♀	♂	♀	♂	♀	♂	♀
Histopathology:								
Thymus involution [§] :	0	0	0	0	1	1	2	2
Liver: hepatocyte vacuolation (incidence /4):								
trace	1	2	2	2	1	2	0	1
minimal	1	1	0	1	2	1	1	0
moderate	0	0	0	0	0	1	3	2
marked	0	0	0	0	0	0	0	1
Liver: lipid periportal depots (incidence /4):								
trace	2	2	3	4	2	2	0	1
minimal	2	1	1	0	1	1	1	0
moderate	0	0	0	0	1	1	3	2
marked	0	0	0	0	0	0	0	1

-The notifier declared:

"On macroscopic examination, a coarse texture of the liver is regarded to be the consequence of the administration of large volumes of vehicle (corn oil). This finding was also seen in control animals and is considered to be a secondary effect following oral intake of high amounts of dietary fat."

As no HCD in dog studies existed where corn oil was the vehicle (no other data available), the position remains speculative. Even if the finding was observed in the study controls (which seems quite acceptable, as corn oil would also lead to a fatty overload in the liver), the treatment seems to exacerbate it slightly.

-For what periportal vacuolation was concerned, no HCD could be submitted, as no former dog studies were available where corn oil had been used as a vehicle. The notifier noted:

"Microscopic findings revealed periportal vacuolation and periportal fat in the liver of males and females which were assessed to be only trace to minimal in severity in control dogs and at 0.04 mg/kg bw/d and 0.4 mg/kg bw/d, respectively. In addition, one incidence of moderate periportal liver vacuolation in mid dose females (but not in males) as well as one incidence each of moderate periportal fat in the mid dose group of males and females was observed. In this context it is important to note that periportal vacuolation and

periportal fat has only been observed in the liver of control males and females of this dog study which used corn oil as the vehicle. No such findings were evident in control dogs of all other studies run around the time when this study was performed and which did not use corn oil as the vehicle. In the absence of treatment-related effects on serum liver enzyme parameters and as periportal vacuolation and periportal fat were also observable in the liver of control males and females of this only study, it is questionable whether one incidence of moderate periportal liver vacuolation in mid dose females as well as one incidence each of moderate periportal fat in the mid dose group of males and females, respectively, may be regarded to be a direct effect of treatment. Since the finding of periportal vacuolation and periportal fat in the liver appears to be strongly related to corn oil administration, the observation of moderate liver hepatocyte vacuolation and fat at the mid dose level is not regarded to be an adverse and toxicologically relevant effect."

RMS had some reservations to dismiss the finding, as cases of moderate or marked fatty vacuolation were not observed in the study controls, although the adversity may indeed be discussed, in the absence of other relevant hepatic or clinico-chemical findings at the mid-dose.

-Concerning the thymus findings the notifier declared:

"The incidence of thymic involution was comparable to the background range for males and not covered by the historical control data in females. However, the single incidences of thymic involution observable in the mid dose group of both sexes are not considered to be toxicologically adverse due to the absence of a correlation to clinical pathology parameters and as absolute and relative organ weights were not affected by treatment. Furthermore, thymic involution has been reported by the pathologists of the testing institute to be quite often seen in dogs and to be a common finding in treated dogs. This effect is in general regarded to be an unspecific response of dogs to stressful situations and does not require a huge amount of stress. Thus, thymic involution may be considered to be not directly related to treatment."

In HCD data provided by the notifier (90d Beagle dog gavage studies, 7 studies 1986-1995), no thymus involution was demonstrated in neither the ♂ nor the ♀ (n=33/29 animals). In non-gavage studies (capsule or diet), 1/48♂ and 0/43♀ (10 studies run in parallel during 1985) exhibited thymus involution. Thus, while spontaneous thymus involution may occur, the average value in the ♂ HCD (2.1%) would not appear to be convincing evidence of a chance event at the lowest dose of the study, but rather be indicative of a treatment-related effect. However, also for this endpoint the adversity may be discussed, in the absence of indications of immunotoxicity in this study.

Therefore, the view of the notifier was not fully supported in the original DAR, and it was considered that thymus involution, liver periportal vacuolation and lipid deposition at 0.4 mg/kg bw/d were compound related, although the adversity of the findings in liver and thymus, and the setting of the NOAEL may be discussed.

For this 13 week oral toxicity study with MITC in dogs, in the DAR the RMS proposed an NOAEL of 0.04 mg/kg bw/day based on thymus involution and liver periportal vacuolation and lipid deposition at the next higher doses. The notifier considered thymus involution not relevant (only a secondary effect due to stress).

However, during peer-review, it was considered that effects at mid-dose level were not marked, but could not be compared with findings from a one year dog study, and with the parent the effects are increasing over time with dosing. The study showed inconsistent findings. However the majority of effects were indicating treatment related effects, consistent at the highest dose tested.

Conclusion, agreed during Peer-Review (PRAPeR 2008):

NOAEL = 0.4 mg/kg b.w./day,

LOAEL = 2 mg/kg b.w./d, based on bodyweight decrease, haematological findings and blood chemistry.

GLP: yes, but no attest of competent authority.

Guidelines: not fully in agreement with Dir 87/302 or OECD 408 (1998-1981).

Deviation from official protocol: Gavage of MITC was performed in two administrations with an interval of 4-5 h. clinical signs were not completely reported.

Material and Methods:

Four pure-bred Beagle dogs/sex/dose received MITC (batch n° BX 340178 AD11308, 95.96% and batch n° BX 340178 AD11328 at 95.96%, formulation CR 20593/L, AD11331) in corn oil by oral gavage, twice daily for 13 weeks at 0.04, 0.4 or 2 mg/kg bw/d. There was an interval of 4-5 hours between the two sub doses. Control dogs received corn oil. Test material was stable over the duration of the study up to week 8 where another aliquot was prepared for week 9. Reduced concentrations of MITC were detected in dosing solutions used in weeks 5 to 8 and to correct this problem, a stock solution was prepared and used in week 10-13.

Test compound BX 340178 AD11308 was used for administration on days 1-35 and day 36-58.

Batch BX 340178 AD 11328 was used on days 58-66.

Batch n° BX 340178, formulation CR 20593/l, AD11331 was used for the remainder of the dosing period from day 59.
The study is accepted.

WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Registration must not be granted on the basis of this document.

B.6.3.4 Summary of short-term toxicity (Annex IIA 5.3)

Drinking water range finding studies were performed for short periods of exposure in mice (6-8 days) and in rats (21 days). The studies were used to set dose levels for the subsequent 90 -day studies.

Rats and mice received metam sodium in drinking water for a 90-day period. Due to the instability of metam sodium in water, the doses of metam sodium actually received were recalculated assuming maximum degradation. In rats, at 18-20 mg/kg bw/d, the olfactory epithelium in the posterior nasal passage was affected and Bowman's glands were prominent and/or vacuolated and disorganized. The NOAEL was set at 0.79 mg/kg bw/d. In mice, at 36-38 mg/kg bw/d, mucosal hyperplasia and epithelial eosinophilia was apparent in the urinary bladder. Nasal cavity was not affected. A NOAEL at 0.49 mg/kg bw/d is proposed.

In a 65-day inhalation study, at 45 mg/m³ metam sodium (12 mg/kg bw/d), rats nasal passages presented a mild degree of mucigenic epithelial hyperplasia as well as lymphocytic rhinitis. Pulmonary histiocytosis and gastric erosions of the stomach were seen in both sexes at 160 mg/m³ (42 mg/kg bw/d). The NOAEL was set at 6.5 mg/m³.

In dogs, metam sodium was given in capsules for 90 days and caused treatment related hepatitis at 5 mg/kg bw/d onwards, resulting in the early termination of two dogs in the top dose group due to adverse clinical signs. Treatment-related hepatitis was associated with progressive inappetence and weight loss. There was a variety of changes, secondary to hepatotoxicity or indicative of generalized toxicity or poor clinical conditions. Considerable reversal of both macroscopic and microscopic changes in the liver had taken place between cessation of treatment with metam sodium and termination of the study. After 1-year, hepatotoxicity was more evident in female dogs as suggested by increased ALT and some liver histopathological findings. The NOAEL was set at 0.1 mg/kg bw/d.

Rabbits did not show systemic toxicity after dermal exposure for 21 days to metam sodium. Local skin reactions such as erythema edema and rhagades and epidermis-dermatitis were seen at 62.5 and 125 mg/kg bw/d. NOAEL for local effects = 31.25 mg/kg bw/d.

As severe toxic effects are reported at doses lower than 50 mg/kg bw/d, RMS considers that metam sodium should be classified R48/22.

According to the company, based on the provisions of EU Directive 2001/59/EC (28th ATP) and taking into account the NOAEL derived in the 90-day rat study, it is principally justified to classify metam with R48/22. However, there was no serious damage or even overt toxicity/mortality seen in the 90-day and/or one-year dog studies. The most pronounced effect in the 90-day study was hepatitis and a drastic increase in liver enzyme parameters.

These effects on liver and liver enzyme parameters were shown to be completely reversible with full recovery observable, which was agreed upon in the evaluation of the RMS. In light of the reversibility of effects on liver and liver enzymes, in the absence of other severe systemic effects and in view of the occurrence of effects in an individual animal only (see metam one-year study), a classification with R48/22 is not considered to be appropriate. Effects mediated by local contact at the site of first contact will be mitigated by consideration of suitable protective equipment and are not within the scope of the R48. Such effects are rather addressed by R phrases applicable for local effects (i.e. R34/35-R38/37/38).

Table B.6.3.4-1: Summary of short term-toxicity of Metam.

Type of test Test species	Test substance purity	Results			References
		NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Symptoms	
90-day drinking water study, mice at 0.018, 0.088, 0.35, 0.62 mg/ml	ref n° Y06930/007 and Y06930/008, 525.54 g/L	0.018 mg/ml = 0.8 mg/kg bw/d	0.088mg/ml = 4.5 mg/kg bw/d	↓ water consumption, hematological changes, histopathological changes in bladder	Whiles, 1991
Oral 90-day drinking water study in rat at 0.018, 0.089 and 0.443 mg/ml	batch n° Y06930/007 and Y03930/008; 525.54 g/L	0.018 mg/ml = 0.5 mg/kg bw/d	0.089mg/ml = 3.1 mg/kg bw/d	↓ body weight, food and water consumption; nasal histopathological findings at top dose	Allen, 1991
Oral 90-day, gavage study in dog at 1, 5 and 10 mg/kg bw/d	Batch n° BAS 005 00N with CTL ref n° Y06930/008 at 43.15%	1 mg/kg bw/d	5 mg/kg bw/d	Clinical signs of toxicity, hepatitis, urinary bladder cell increased mitosis	Brammer, 1992
Oral 1-year dog study by gavage at 0.05, 0.1 or 1 mg/kg bw/d	batch n° BAS/005/00N 90-2; CTL n° Y06930/008; 525.54 g/L (43.148% w/w)	0.1 mg/kg bw/d	1 mg/kg bw/d	Hepatotoxicity (blood chemistry and histopathological findings)	Brammer, 1993
Inhalation 90-day rat study at 6.5, 45, 160 mg/m ³	Vapam technical: lot n° EHC 0355-21; containing 32.7% metam sodium.	Metam: 65 mg/m ³ = 1.75 mg/kg bw/d	45 mg/m ³ = 12 mg/kg bw/d	Liver and nasal toxicity	Knapp, 1983
Percutaneous 21 day rabbit study at 31.25, 62.5 or 125 mg/kg bw/d	BAS 00500 N 42.4%	NOAEL local effects = 31.25 mg/kg bw/d NOAEL systemic effects	62.5 mg/kg bw/d >125 mg/kg bw/d	Local dermal effects: erythema, edema, rhagades; epidermodermatitis No systemic toxicity.	Leuschner, 1979

A 4-week inhalation rat study was performed with MITC. At doses of 100 mg/m³, lung weight was increased, and was associated with bronchopneumonia and epithelial proliferation in bronchi and bronchiole. At this dose level, proliferation in tracheal epithelial cells and inflammatory changes in the nasal cavity and atrophy of the olfactory epithelium as well as focal metaplasia of squamous epithelial cells in the area of respiratory epithelium were reported. The company proposed a NOAEL = 20 mg/m³ (5.76 mg/kg bw/d). A NOAEL for systemic effects is proposed at 5 mg/m³.

In a 90-day dog study by gavage with MITC, at the dose level of 2 mg/kg bw/d body weight decrease, haematological findings and blood chemistry were observed.

Table B.6.3.4-2: Summary of short term-toxicity of MITC

Type of test	Test	Results	References
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Test species	substance purity	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Symptoms	
MITC: 4-week inhalation study in rats at 5, 20, 100 mg/m ³	Batch n° 6205MK, 96.9%	5 mg/m ³ (1.7 ppm)	20 mg/m ³ = 6.8 ppm	Nasal cavity atrophy	Klimisch, 1987
MITC: 90-day dog study gavage at 0.04, 0.4 or 2 mg/kg bw/d	Batch n° BX340178 AD11308, 95.96% and batch n° BX 340178 AD11328 at 95.96% formulation CR 20593/l, AD11331	0.4 mg/kg bw/d	2 mg/kg bw/d	body weight decrease, haematological findings and blood chemistry	Harling et al, 1986

B.6.4a Genotoxicity studies with metam sodium (Annex IIA 5.4)

B.6.4.1a *In vitro* genotoxicity testing (Annex IIA 5.4.1)

B.6.4.1.1a Gene mutation test in bacterial cells (Annex IIA 5.4.1)

- Report on the Study of Metam Sodium in the Ames test (Engelhardt, 1987)

Findings:

Bacteriotoxicity was observed depending on the strain, experiment and test conditions from about 100 µg-2500 µg/plate onwards. No increase in mutagenicity was reported.

Conclusion:

According to the experimental results, it can be concluded that metam sodium is not mutagenic under these experimental conditions.

GLP status: yes (no attest of national authority).

Guidelines: experimental protocol in agreement with Directive 2000/32/CE Annex 4D or 92/69, 84/449 or OCDE 471 (1997-83).

Material and methods: metam sodium (batch n° ZH 130585, test substance n° 85/232; 42.2%) in water was tested at 4 up to 5000 µg/plate towards strains TA92, TA98, TA100, TA1535, TA1537, TA1538 using the standard plate incorporation assay or the preincubation test (20 min at 37°C). S9 was prepared from Arochlor 1254 pretreated rats. The stability of metam sodium was proved analytically. Positive controls were mitomycin, 2 aminoanthracene, N-methyl-N-nitrosoguanidine or 4-nitro-o-phenylenediamine or 9 aminoacridine. The study is accepted.

- Ames test for N-methyldithiocarbamate (sodium salt) (Oesch, 1979)

Findings:

Metam sodium was tested at 1.6, 5.1, 16, 51, 160, 510 or 1600 µg/plate with or without S9 mix. In the absence of S9 mix, metam sodium was markedly toxic at a dose of 160 µg/plate. Much less, toxicity was observed in the presence of S9 mix. No mutagenic activity was observed under any experimental condition.

Conclusion: metam sodium was not mutagenic under these experimental conditions.

GLP status: no

Guidelines: experimental protocol not fully in agreement with 2000/32/CE Annex 4D or 92/69, 84/449 or OCDE 471 (1997-83).

Deviation from official protocol: strains were not checked for their characteristics; test performed in duplicate and not repeated. Neither means nor standard deviations were provided. Criteria for accepting the test are not provided.

Material and methods: metam sodium (purity, batch n°?) was dissolved in DMSO strains TA100, TA1535, TA1537, TA1538 were used. The test compound was assayed at various doses up to 1600 µg/plate in the plate incorporation test with and w/o S9 mix from Arochlor 1254 pretreated rats. Positive controls were benzopyrene, 2 aminoanthracene, N-methyl-N-nitrosoguanidine or 3-methylcholanthrene. The study is not accepted, as additional information as the purity and batch n° was unknown.

B.6.4.1.2a Gene mutation test in mammalian cells (Annex IIA 5.4.1)

- Report on the study for gene mutations *in vitro* of Metam sodium in Chinese Hamster Ovary Cells (HGPRT locus) with and without metabolic activation (Engelhardt, 1987)

Findings:

Metam sodium was tested three times at the same eight dose levels. The experiment was repeated because in the first study, the cloning efficiency was too low and in the second experiment, the positive control resulted in a lower mutant yield than usual. These two experiments did not show a mutagenic response according to the evaluation criteria defined. In the third study, 0.001 mg/ml lead to significantly increased n° of mutants in the presence of metabolic activation. However, since this was a singular event and no dose response relation was observed, it is not taken as evidence of mutagenicity. A borderline effect was observed at 0.01 mg/ml, top dose used, showing also cytotoxicity.

Table B.6.4.1.2a CHO cells- HGPRT test.

Concentration mg/ml	Experiment 1/2/3	
	N° mutants/10 ⁶ cells	Cytotoxicity (at transition into selection medium) %
No metabolic activation		
0	0/0/0	51.50/56.25/93.25
0.0000464	0/0/0	49.5/64.25/76.75
0.0001	0/0/0	42.25/65.25/75.75
0.000215	0/0/0	56/54/64.5
0.000464	11/16/0	47.25/69.2/80.55
0.001	0/0/0	57/60.5/72
0.00215	0/0/0.92	55.25/60.7/73.5
0.00464	0/0/0	55/66.25/68
0.01	0/0/8.5	47.5/56/62.5
Positive control: EMS	817/633/250	27/30/69.5
With metabolic activation		
0	0/0/0	43.7/88/97
0.0000464	0/0/0	48.25/54/80
0.0001	6.67/3.77/0.68	48.75/53/99
0.000215	0/2.82/8.55	39/47.25/78
0.000464	0/0/0	37/60.5/78
0.001	9.33/0/34.58	36.25/58.25/86
0.00215	0/0/0	54.75/73.75/76
0.00464	5.33/0	41.5/72.25/91
0.01	13.33/6.56/13.95	49.7/61/86
Positive control: MCA	50/79/725	46.7/47.5/79

Conclusion:

A genotoxic effect at cytotoxic dose levels cannot be ruled out completely: however, the results were not dose-related and none of the negative controls registered above a mutation rate of zero, a situation that makes one suspect that occurrence at other doses is random. Nonetheless, equivocal mutagenicity in the absence and presence of a metabolic activation system cannot be discounted.

Comments from the company: The notifier re-evaluated the results of this study and concludes that metam sodium dose not induce forward mutations at the HPRT locus in CHO cells in both the presence and absence of metabolic activation.

This conclusion is supported by the absence of a clear-dose response relationship evident and a slightly positive response (i.e. increased mutation rate) was only evident at the highest dose level where also significant cytotoxicity was noted. In addition, the pre-defined laboratory criteria for a positive response in the HPRT-assay were not fulfilled. The absence of a(n) (overall) genotoxic effect is also confirmed by the results of *in vivo* mutagenicity/genotoxicity testing of metam sodium where no positive response was observed (please refer to B.6.4.2a *In vivo* genotoxicity testing (somatic cells) - Metam sodium: an evaluation in the mouse micronucleus test (*in vivo*) (Barber and Mackay, 1996) and Cytogenetic study *in vivo* of Metam-Sodium in Chinese hamsters bone marrow chromosome analysis, single oral administration (Gelbke and Engelhardt, 1987)).

GLP status: yes (no attest of national authority).

Guidelines: experimental protocol not fully in agreement with Directive 2000/32/CE Annex 4A or 87/302 or OECD 476 (1997-84).

Deviation from official protocol: too low cell number; absence of mycoplasma not controlled. Relative cytotoxicity is not given.

Material and methods: metam sodium (batch n° ZH 130 585; 42.2% purity)

CHO cell line sub-strain K1 was used.

Range finding test: cytotoxicity test was performed using 50 mg test substance/ml in the absence of S9 activation for an exposure period of 16 h. visible precipitation occurred at 1 mg/ml and decrease of cloning efficiency at 0.001 mg/ml.

100 000 cells were seeded per flask for an attachment period of 24 h. Test solution was prepared in culture medium without FCS and diluted with culture medium. Exposure period was 4 hr. 20 hr after treatment termination 200 cells of each dose were taken in duplicate and allowed for colony formation. A second assay was carried out following the same procedure at the transition into the selection medium. Expression period was of 8 days. 4-sub culturing was used during the expression period. At the last passage, the cells are transferred into the 6-TG containing selection medium and 300 000 cells are seeded into the selection medium, 5 flasks/dose.

Study initiation: 10⁵ cells were seeded in flasks (duplicate), covered with medium, and left for attachment period of 24 hr. Then the medium was changed and test material as well as S9 was added where needed and the mixture was left for 4 hr with and without S9 mix. After termination, the monolayer was rinsed. Expression time of 8 days: 16 hr after termination of exposure, cells were subcultured (all cells of same dose level were pooled and reseeded at density of 10⁵/flask in duplicate). 200 cells of each dose level in duplicate were used for

cloning efficiency determination. Further sub culturing was carried out 2 days later and twice in the following week, the last passage was transferred into 6-TG-containing selection medium (3×10^5 cells/flask; 5 flasks/dose; all cells of each dose are obtained from pooled cells of the 2 flasks/dose). A cloning efficiency was also performed at this point, seeding 200 cells in duplicate. 3-methylcholantrene or EMS was used as positive control. Acceptance criteria are well described and correct. The study is accepted.

B.6.4.1.3a *In vitro* mammalian cytogenetic test studies (Annex IIA 5.4.1)

- Report on the *in vitro* cytogenetic investigations in human lymphocytes with metam sodium (Gelbke and Engelhardt, 1987)

Findings:

According to the results presented in table B.6.4.1.3-1a, metam sodium caused a statistically significant and dose-dependent increase in the number of chromosomally damaged cells with or without S9 mix.

Table B.6.4.1.3-1a: Chromosomal analysis of human lymphocytes exposed to metam sodium

dose µg/ml	Mitotic index % Rel	N° metapha ses analyzed	Metapha ses with aberration including gaps%	Metaphases with aberration excluding gaps %	N° cells with exchange	Multiple aberrant met./pul verizatio n	Polyploidy %
- S9mix							
control	100	200	5	1	0	0	0.5
solvent	100	200	5	0.5	0	0	0
20	143	100	24*	10*	2	0	0
10	161	200	12.5	3.5	0	0	0
5	144	200	8.5	1	0	0	0
1	104	200	8.5	1.5	0	0	0.5
Mito: 0.3	27.7	200	53*	51*	24*	9/0	0
+ S9mix							
control	100	200	5.5	1	0.5	0	0
solvent	100	200	5	0.5	0	0	0.5
40	157	200	20*	8.5*	1	0	0
20	117	200	14*	5*	1	0	0
10	100	200	10.5	2	0	0	0
Cyclo:6	84	200	23*	15*	4*	0	0

* Statistically significantly different from control

Conclusion:

Metam sodium has a clastogenic effect *in vitro* using human lymphocytes.

Comment from the company: Based on the results obtained, metam sodium appears to be clastogenic in human lymphocytes *in vitro* both in the presence and in the absence of a metabolic activation system. However, the slides and the results were re-examined taking into account the morphology of the chromosomes. It could be demonstrated that at 10 and 20 µg/mL (without activation) and at 40 µg/mL (with activation) severe toxic effects on the chromosome morphology was evident preventing an accurate evaluation of the chromosome damaging potential. Slides were therefore not suitable for chromosomal aberration analysis. The results of the re-examination of the slides showed that metam-sodium does not reveal clastogenic activity in the absence and presence of metabolic activation at concentrations which do not exert severely cytotoxic effects on chromosomal morphology and therefore do not prevent an accurate evaluation of structural chromosomal aberrations. The absence of a clastogenic potential of metam in this *in vitro* test is also supported by the results of *in vivo* mutagenicity/genotoxicity testing of metam sodium where no positive response was observed and which confirms the absence of an overall mutagenic/genotoxic potential of metam sodium (please refer to B.6.4.2a *In vivo* genotoxicity testing (somatic cells) The small but statistically significant increase in the number of structural chromosomal aberrations at 20 µg/mL in the presence of metabolic activation was only slightly outside the range of historical controls of the testing laboratory (0.0 – 4.0 % aberrant cells) and was thus,

considered to be not of toxicological and/or biological significance. Finally, the study was conducted in 1986 when the effects of physiological disturbances (e.g. pH and osmolality changes) and toxicity on chromosomal morphology were not well understood. The understanding of the result of such toxic (rather than genotoxic) effects on chromosomes had developed significantly since 1986 and a concentration inducing such severe cytotoxic effects on chromosome morphology would not now be selected or even required for chromosomal aberration analysis in this assay type (Mackay, J.M. (1996) Review of Metam-sodium Genetic Toxicology; Central Toxicology Laboratory, Macclesfield, Cheshire, UK; Report No. CTL/P/5287; not published).

GLP status: no

Guidelines: experimental protocol not fully in agreement with Directive 2000/32/CE Annex 4A or 92/69-84/449 or OECD 473 (1997-83).

Deviation from official protocol: data from pretest not provided. Abnormal metaphases were counted but the number of abnormalities is not reported. Exposure time is too long (OK for dir 84/449).

Material and methods: The doses of metam sodium (batch n° ZH 130585, test substance n° 85/232 AT 42.2%) dissolved in water were determined from a pretest with cultures exposed to a wide dose range. Due to the findings, the doses selected were: 20µg/ml (w/o S9 mix) and 40µg/ml with S9 mix and this selection was based on the quality of the metaphases and not on the mitotic index as required in the test guideline. The reason for this is that the test substance concentration causing reduction of the mitotic index is at dose levels that severely affect the chromosomes, thus no longer allowing evaluation. 10µg/ml and 5µg/ml w/o S9 mix and 10µg/ml with S9 mix were chosen as further doses. In addition, one higher and one lower dose were selected. Positive controls were cyclophosphamide and mitomycin. 100 metaphases of each exposed culture were analyzed for CA. Whole blood was added to the culture medium and incubated for about 48 h; solutions of test substances were then added and subsequently re-incubated for further 24 h. Colcemid was added 2-3 hr before harvesting the dividing human lymphocytes. For assays with S9, 2 hr of incubation were performed and cells were washed and re-incubated for further 22 hr. a mitotic index based on 1500 cells was determined for at least the 2 top doses and for the solvent and negative controls. Fisher's exact test was used. The stability of test substance was proved. S9 was from Arochlor 1254 pretreated rats. The study is accepted.

- Metam sodium: *in vitro* cytogenetic assay in human lymphocytes (Barber and Mackay, 1996)

Findings:

Concentration related reductions in mitotic activity were observed in the cultures, thus demonstrating that metam sodium is biologically active in this test system. No statistically or biologically significant increases in the percentage of aberrant cells, compared to the control value, were recorded for any cultures treated with metam sodium in the absence of S9mix.

A small but statistically significant increase in the percentage of aberrant cells, compared to the solvent control value, was recorded in cultures treated with metam sodium at 40µg/ml in the presence of S9mix. In order to further investigate this small increase, extended analyses of an additional 100 cells in metaphase/culture were conducted for the + S9 portion of this study. A small but statistically significant increase in the percentage of aberrant cells in cultures treated with metam sodium at 20 and 40µg/ml is noted. These increases are however within the laboratory solvent control range (0.0-5.5% aberrant cells, n= 222, over a period of approximately 3 years). The statistical significance could be due to the low solvent control value in this portion of the study rather than to any *in vitro* clastogenic activity of metam sodium. The small increases observed in this study are, according to the study director, therefore considered to be of no biological significance.

The sensitivity of the test system and the metabolic activation system were clearly demonstrated by the increases in percentage of aberrant cells induced by the + control agents.

Table B.6.4.1.3-2a: chromosomal analysis of human lymphocytes exposed to metam sodium

Dose µg/ml	Mitotic index %		Mean % aberrant cells excl. gaps		Aberrations/cell excluding gaps	
- S9mix (100 metaphases/culture)						
Solvent control	12.9		0.0		0.00	
30	5.6		0.5		0.005	
20	13		0.5		0.015	
2.5	10.1		0.5		0.005	
Mitomycin 0.2 µg	6.2		56*		1.16	
+ S9mix						
N° metaphases/culture:						
	100	200	100	200	100	200
Solvent control	9.7	9.7	0.0	0.25	0.0	0.003
40	3.9	3.9	3.00*	2.75*	0.03	0.028
20	5.9	5.9	1.50	1.75*	0.015	0.02
5	9.0	9.0	1.00	0.50	0.01	0.005
Cyclophosphamide 50 µg	4.3	4.3	40*	36*	0.44	0.44

* Statistically significantly different from control

Conclusion:

RMS considers that, although the increase in aberrant cells is low, the repeat study produced again very slight positive results. Under these experimental conditions, the results should be considered as equivocal.

GLP status: the study is GLP (no attest of national authority) except for stability/homogeneity/concentration of active ingredient with or without solvent.

Guidelines: experimental protocol not fully in agreement with Directive 2000/32/CE Annex 4A or 92/69-84/449 or OECD 473 (1997-83).

Deviation from official protocol: data from pretest not provided. Exposure time with S9 is too short. Collecting time at 72 h., abnormal metaphases were counted but the number of abnormalities is not reported.

Material and methods: duplicate human peripheral blood cultures from a male donor were exposed to metam sodium (batch n° WCR 14989-13-01; CTL ref Y06930/013; 32.3% purity) solubilized in water at 40, 20 or 5 µg/ml + S9 (from Arochlor 1254 pretreated rat liver) mix and at 30, 20, or 2.5 µg/ml without S9 mix. The cultures were harvested at 72 hr after culture initiation. Cultures treated in the presence of S9 mix were treated for a period of approx. 2 hr at 37°C and were, after removal of culture medium re-incubated for the remainder of the 72 h. 2 hr prior harvesting, the cultures were treated with colcemid. Positive controls were mitomycin and cyclophosphamide. No significant increase in osmolality or changes in pH were seen. The study is accepted.

B.6.4.1.4a Testing for *in vitro* DNA damage (Annex IIA 5.4.1)

- Report on the mutagenicity test on metam sodium in the rat primary hepatocyte unscheduled DNA Synthesis assay (Cifone, 1987)

Findings:

Treatments above 1000 µl/ml appeared lethal (17.3% survival). When the concentration was reduced to 500 µl/ml, survival increased to 55%. At 50 µl/ml, cell morphology was similar to the controls and survival increased to 93%. The positive control 2-AAF was essentially non-toxic in this trial. The minimum criteria for UDS in this assay were a mean net nuclear grain count exceeding 7.71 or at least 16% of the nuclei containing 6 or more grains, or at least 2% of the nuclei containing 20 or more grains.

None of the treatments with metam sodium caused nuclear labeling significantly different from the negative control. In contrast, 2-AAF treatment induced large increases in nuclear labeling that greatly exceeded all three criteria used to indicate UDS.

Table B.6.4.1.4-1a: UDS in hepatocytes.

Concentration nl/ml	UDS grains /nucleus	Mean% nuclei with >6 grains	Mean% nuclei with >20 grains	Survival at 21 h %
Control	1.71	6	0	100
2-AAF	9.57	69	9.3	98.5
Metam:				
250	2.38	12	0	Cells died late
100	0.49	0	0	17.3
50	0.44	0	0	55.3
10	2.05	9.3	0	83.7
5	1.28	4.7	0	93.4
2.5	1.02	1.3	0	96
1.0	2.69	10.7	0	102
0.5	0.75	0.7	0	96

Conclusion:

Metam sodium did not induce significant changes in the nuclear labeling of primary rat hepatocytes for an applied concentration range of 250nl/ml to 0.5nl/ml.

GLP status: limited to an attest of GLP/QAU.

Guidelines: experimental protocol not fully in agreement with Directive 87/302/EEC Annex V B or OECD 482 (1986).

Deviation from official protocol: experiment was not repeated.

Material and methods: metam sodium (test substance n° 85/232; batch n° ZH 130 585, at 42.2%) was solubilized into WME medium with 1% serum and 15 concentrations were prepared ranging from 1000 nl/ml to 0.025 nl/ml. 8 treatments from 250nl/ml to 0.5nl/ml were selected for the analysis of nuclear labeling. Hepatocyte cells were exposed for 18 hr by replacing the media on the cells with the media containing the appropriated concentration of test material. Viability of 81.5% was measured when the study started. The viable cells attached to the culture dishes during 1.5 hr and the treatments were initiated one ½ h later with cell monolayer that were 92% viable. After an additional 21 hr in culture, the average viable cells in the negative controls were 95.6% of the viable count at starting of treatments.

The study is accepted.

- Report on the mutagenicity test on metam sodium in the Rec-Assay with *Bacillus subtilis* (Hoorn et al, 1987)

The rationale of the *B. subtilis* rec-assay is based on the relative difference of survival of a DNA repair-recombination proficient strain and its deficient strain, which is interpreted as genotoxicity.

The *bacillus subtilis* strain H17 (rec⁺) and M45 (rec⁻) were used in this study. Both strains are recombination efficient and deficient strains. The damage to the strain is repaired through cellular recombination functions in wild type (rec⁺) cells but not in recombination less mutant (rec⁻) cells. It is highly probable that such DNA damage-provoking agents are mutagenic. Strain M45 carries two independent rec mutations and is sensitive to both frameshift and base-change mutagens.

Findings:

Positive controls showed the expected inhibition zone. Metam sodium did not exhibit genetic activity in any of the assays conducted in this evaluation and was not recombinogenic to *Bacillus subtilis* indicator organisms under these test conditions.

Conclusion:

Metam sodium is not recombinogenic to *Bacillus subtilis* indicator organism under these experimental conditions.

GLP status: only attest of study director.

Guidelines: experimental protocol not reported in EC Directive.

Material and methods: metam sodium (batch n° ZH 130585; BAS 005 00 N/metam sodium at 510 g/L; 42.2%) was used at doses ranging from 0.1 µl to 150 µl/plate. Each assay was conducted in triplicate. Strain H17 and M45 were used with or without S9 metabolic activation system from Arochlor pretreated rat liver. The solvent water was negative control and MMS and sterigmatocystin were used as positive controls. The test is a plate incorporation assay and the test substance diffuses from a center well to the surrounding agar media. Strain M45 is unable to repair damaged DNA and the zone of inhibition is measured. Any test zone in which a differential of 4 mm or greater occur will be considered to have recombinogenic activity. The assay was repeated.

The study is accepted.

B.6.4.2a *In vivo* genotoxicity testing (somatic cells) (Annex IIA 5.4.2)

B.6.4.2.1a *In vivo* micronucleus test (Annex IIA 5.4.2)

- Metam sodium: an evaluation in the mouse micronucleus test (in vivo) (Barber and Mackay, 1996)

Findings:

It is mentioned that adverse reactions to treatment were observed for both males and females but the results are not reported.

A single oral dose of metam sodium was given to mice at a dose level of 500 mg/kg bw, the MTD. No statistically or biologically significant increases in the incidence of micro-nucleated polychromatic erythrocytes over the solvent control were seen in either sex at either of the sampling times investigated. The positive control, cyclophosphamide induced statistically significant meaningful increases in MN demonstrating the sensitivity of the test system.

Conclusion:

Metam sodium, under these experimental conditions, is not clastogenic in the mouse MN test.

GLP status: the study is GLP (no attest of national authority) except for stability/homogeneity/concentration of active ingredient with or without solvent

Guidelines: experimental protocol not fully in agreement with Directive 92/69-84/449/CE Annex 4C or OECD 474 (1997-83).

Deviation from official protocol: peripheral red blood cells were not examined; this is in agreement with Dir 92/69. Toxicity data not reported.

Material and methods: 5 CD mice/sex received a single oral dose of metam sodium (batch n° WRC 14989-13-01; purity of 32.3%) in water at 500 mg/kg bw. Bone marrow samples were taken 24 and 48 hr after dosing. Cyclophosphamide was used as positive control. Analysis of variance was used for statistical analysis.

A preliminary study was performed on two mice/sex/dose which received 800, 1250 or 2000 mg/kg. Groups of five mice/sex were then dosed with metam sodium at 800, 1250 and 500 mg/kg. Based on patterns of clinical signs and lethality, a dose level of 500 mg/kg was selected.

The study is accepted.

B.6.4.2.2a *In vivo* mammalian chromosome aberration test (Annex IIA 5.4.2)

- Cytogenetic study *in vivo* of Metam-Sodium in Chinese hamsters bone marrow chromosome analysis, single oral administration (Gelbke and Engelhardt, 1987)

Findings:

In a pretest for determination of acute oral toxicity, deaths were observed down to a dose of 1470 mg/kg bw. The amount at which all animals survived was 1210 mg/kg bw, but it led to signs of toxicity such as irregular breathing and squatting posture after test substance administration. In some cases, apathy was observed and the general state of some animals was poor. 1200 mg/kg was considered as the highest suitable dose. However, 8/20 animals died after this dose level and it was decided to reduce the top dose down to 900 mg/kg bw. Again, a few animals died, moreover, the slides were prepared from the remaining hamsters and could not be evaluated due to a pure quality of the chromosome and/or due to the fact that only, and few cells were found. To find out whether the results obtained with the 900 mg/kg were incidental or influenced by metam sodium, an additional study using four control and six treated hamsters with 900 mg/kg was carried out. This experiment confirmed the above-mentioned findings. Therefore, a dose of 600 mg/kg was administered as the highest dose.

Metam sodium dissolved in water was administered once orally at 600, 300 and 150 mg/kg bw and led to irregular respiration about 30 minutes after the administration and depending on the dose for the duration of about 1-2 hours.

The single oral dose of metam sodium did not lead to aberrant metaphases; there were no significant differences in the type and frequency of aberrations between the treated animals and controls. The statistical significance observed in the 600 mg/kg group at 24 hr is due to the low spontaneous rate of 0.1% aberrant metaphases excluding gaps and is of no biological significance since 0.9% is in the range of that of the historical control data.

Type of aberrations such as exchanges, multiple aberrant cells or disintegration of chromosomal structure was not found.

Table B.6.4.2.2-1a: experimental results in bone marrow of Chinese hamsters exposed to metam sodium

Test group n°/ Dose mg/kg	N° animals, with aberrant metaphase	Sacrifice time	Metaphases including gaps %	Metaphases excluding gaps %	Polyploidy N° cells
1/Negative control	9/10	24 hr	2.6	0.1	3
Metam sodium:					
2/600 mg/kg	7/10	6 hr	1.4	0.3	17
3/600 mg/kg	9/10	24 hr	2.4	0.9*	18
4/600 mg/kg	5/10	48 hr	0.7	0.1	8
5/300 mg/kg	8/10	24 hr	1.8	0.2	23
6/150 mg/kg	6/10	24 hr	1.2	0.2	7
7/Cyclophosphamide 40 mg/kg	10/10	24 hr	32.2*	26.1*	11

Conclusion:

Metam sodium does not have chromosome-damaging effect under the experimental conditions.

GLP status: yes (no attest of national authority)

Guidelines: experimental protocol not fully in agreement with Directive 2000/32/CE Annex 4C or 92/69-84/449/CE or OECD 474 (1997-83).

Deviation from official protocol: mitotic index was not measured (in agreement with Dir 92/69). Abnormal metaphases were counted but not abnormalities. Toxicity data were not reported.

Material and methods: Five Chinese hamsters SH(chin)Ki strain /sex/dose received a single oral dose of metam sodium (batch n° ZH130585; test substance n° 85/232 at 42.2%) dissolved in water. The stability of the test substance was proved. Cyclophosphamide was used as positive control. Animals were sacrificed and bone marrow was prepared 6, 24 and 48 hr after dosing with 600 mg/kg. Animals dosed with 300 or 150 mg/kg, positive and negative control; the 24 hr intervals were only investigated. Colcemid was administered 2 hr before sacrifice. 100 metaphases were analyzed per animal.

The study is accepted.

Already Peer Reviewed, EFSA 2008

The genotoxicity testing and potential of metam was discussed during peer review.

-A slightly positive response in the HPRT test was observed at a single dose in one of three tests (however, dose-response was lacking, making the significantly increased incidences equivocal);

-In the *in-vitro* chromosomal aberration tests in human lymphocytes were slightly positive to equivocal. In a first study (1987), chromosomal aberrations were slightly induced at 20 µg/mL (10%*, -S9; 5%*, +S9) and at 40 µg/mL (8.5%*, +S9). In a second study (1996), aberration incidence was higher at 20 µg/mL (1.75 %*) and at 40 µg/mL (2.75*-3*%), only in the presence but not in the absence of S9. While the response in the first study exceeded the time-matched HCD (0-4%), this was not the case for the most recent study (0-5.5%). Whereas a slight clastogenic potency *in-vitro* could not entirely be discarded, it was of note that the events were observed only in cytotoxic conditions, in so far that scoring of chromosomes was almost impaired.

-Negative Ames test and *in-vitro* UDS and Rec assay were reported.

-*In-vivo*, the MN and CA assays were also negative. The only effect, which could be considered treatment-related, was the a slightly increased incidence of 'polyploid' cells in the Chinese Hamster bone marrow cells, amounting to 3/7/23/18 at 0/150/300/600 mg/kg. The effect was relevant at the two highest doses, corresponding to about $\frac{1}{3}$ - $\frac{2}{3}$ of the LD₅₀ in the rat. In the absence of chromosome-damaging capacity *in-vivo*, the genotoxicological significance of the finding remained unknown, but the event was suspected to be the result of cell toxicity.

RMS considers that the negative result observed in the *in-vitro* UDS test does not support the need for an additional test *in-vivo*. As the *in-vivo* UDS test is not particularly sensitive, and other *in-vivo* tests are negative, it is unclear which other assay could provide more relevant information.

Conclusion: The overall weight-of-evidence suggests that Metam-sodium *in-vivo* is devoid of genotoxic potential.

B.6.4b genotoxicity studies with MITC (Annex IIA 5.4)

B.6.4.1b *In vitro* genotoxicity testing (Annex IIA 5.4.1)

B.6.4.1.1b Gene mutation test in bacterial cells (Annex IIA 5.4.1)

- **Report on the study of Methylisothiocyanate in the Ames test (Gelbke and, Engelhardt, 1986)**

Findings:

A bacteriotoxic effect was seen at dose $\geq 500 \mu\text{g/plate}$. Number of revertants was not increased with or without S9 mix.

Conclusion:

MITC was not mutagenic under these experimental conditions.

GLP status: yes (no attest of national authority).

Guidelines: experimental protocol in agreement with Directive 2000/32/CE Annex 4D or 92/69, 84/449 or OCDE 471 (1997-83).

Material and methods: MITC (batch n° 6205MK; test substance n° 85/231; 98%) solubilized in DMSO was tested at doses ranging from 20 up to 5000 $\mu\text{g/plate}$ towards strain TA98, TA100, TA1535, TA1537, TA1538 using the standard plate incorporation assay. Stability of MITC was tested throughout the study. S9 was obtained from Arochlor 1254 pretreated rats. Positive controls were 2 aminoanthracene, N-methyl-N-nitrosoguanidine or 4-nitro-o-phenylenediamine or 9 aminoacridine. The study is accepted.

B.6.4.1.2b *In vitro* mammalian cytogenetic test studies (Annex IIA 5.4.1)

- **Report on the *in vitro* cytogenetic investigations in human lymphocytes with Methylisothiocyanate (Engelhardt and Gelbke, 1987)**

Findings:

MITC treatment did not significantly increase the number of aberrant metaphases excluding gaps above that observed in negative controls, in either the presence or absence of metabolic activation. However, in the presence of metabolic activation, although not statistically significant and not dose-related, there was an increase in the number of aberrant metaphases (excluding gaps) above control levels at all tested doses. In both the presence and absence of metabolic activation, the number of aberrant metaphases (including gaps) was increased at all concentrations tested. This effect was not dose-related.

Table B.6.4.1.2-1b: chromosomal analysis of human lymphocytes exposed to MITC on 200 metaphases (except for positive controls: 100 metaphases).

Dose µg/ml	Mitotic index % relative	N° cultures with aberrant metaphases	Metaphases with aberration including gaps %	Metaphases with aberration excluding gaps %	Exchange %	Polyplody %
- S9mix						
control		2	5	1	0	0.5
DMSO	100	2	5	3.5	0	0.5
0.5	55.8	2	13.5*	2	0	0
0.1	71.6	2	8	3.5	0	0.5
0.05	75	2	11.5	2.5	0	1
0.3 mito	34	2	53*	51*	24*	0
+ S9mix						
control		2	5.5	1	0.5	0
DMSO	100	2	3.5	0.5	0	0.5
1	89	2	14.5*	3	0	0.5
0.5	141	2	8	2	0	1
0.1	130	2	10	3	0	0.5
Cyclo 6	130	2	23*	15*	4*	0

* Statistically significantly different from control

Conclusion:

MITC has no chromosome-damaging effect in vitro using human lymphocytes under these experimental results.

GLP status: limited to an attest of GLP/QAU.

Guidelines: experimental protocol not fully in agreement with Directive 2000/32/CE Annex 4A or 92/69-84/449 or OECD 473 (1997-83).

Deviation from official protocol: data from pretest not provided. Doses are selected taking into account of chromosomal toxicity. Exposure time without S9 is too long (in agreement with dir 84/449). Abnormal metaphases but no number of abnormalities was reported.

Material and methods: human venous blood cultures were exposed to MITC (batch n° 6205MK; test substance n° 85/231; 98.1% purity) dissolved in DMSO (0.1 ml) at 1, 0.5 or 0.1 µg/ml. S9mix (from Arochlor 1254 pretreated rat liver) mix and at 0.5, 0.1 or 0.05 µg/ml without S9 mix.. Cultures treated in the presence of S9 mix were treated for a period of approx. 2 hr at 37°C and were, after removal of culture medium re-incubated for further 22 h. Assays without S9 were incubated for 24 h. 2-3 hr prior harvesting, the cultures were treated with colcemid. The whole duration of incubation was 72 h. Positive controls were mitomycin and cyclophosphamide. The stability of MITC was controlled. 100 metaphases of each culture were analyzed for chromosomal aberrations. for positive controls, 50 metaphases/culture were examined. Mitotic index based on 1500 cells was determined for at least the top two doses and for controls. The study is accepted.

B.6.4.1.3b testing for *in vitro* DNA damage (Annex IIA 5.4.1)**- Report on the mutagenicity test on MITC in the recombination assays with *bacillus subtilis* strains H17 (rec⁺) and M45 (rec⁻) (Jagannath, 1986)**Findings:

This test uses recombination efficient (Rec⁺) and deficient (Rec⁻) strains of *bacillus subtilis*. The damage to DNA is repaired through cellular recombination functions in wild type cells (Rec⁺) but not in recombination less mutant cells (Rec⁻). Most agents showing increased lethal activity on Rec⁻ over Rec⁺ cells have damaged the DNA. It is highly probable that such DNA damage-provoking agents are mutagenic. Strain M45 carries 2 independent Rec mutations and is sensitive to both frameshift and base-change.

The results of the initial and independent assays conducted on MITC at dose levels from 1 to 10000 µg/plate with or without metabolic activation system did not exhibit significant difference in the zone of inhibition between the two strains. The test material was toxic to both strains at 5004 µg/plate onwards.

Positive controls induced significant increases in the zones of inhibition with the M45 culture, which demonstrated the effectiveness of the system.

Table B.6.4.1.3-1b: REC assay with *bacillus subtilis*

Dose (µg)/plate	Differential inhibition of M45-H17
-----------------	------------------------------------

	-S9 mix	+ S9mix
1	0.0	0.0
10	0.0	0.0
100	0.0	0.0
500	0.0	0.0
1001	0.0	0.0
2502	0.0	0.0
5004	-0.6	-1.0
10008	0.0	0.0
Solvent control	0.0	0.0
Negative control	-0.3	0.0
Positive control	7.7	4.6

Conclusion:

MITC has no DNA damaging effect under these conditions according to the evaluation criteria.

GLP status: yes (no attest of national authority).

Guidelines: no EU or OECD protocol.

Material and methods: bacterial strains H17 Rec⁺ (wild type strain) and M45 Rec⁻ were exposed to MITC (identification n° n85/231 charge 6205MK, purity?) dissolved in DMSO at 1, 10, 100, 500, 1001, 2502, 5004 or 10008 µg plate with or without liver metabolic activation system from Arochlor pretreated rats. Negative controls were solvents. Positive control was methylmethanesulfonate and diethylnitrosamine. The assay is a plate incorporation assay, where the chemical diffuses from a center well to the surrounding agar media inhibiting the growth of the bacteria. Any test in which a differential of 4 mm or greater has occurred is considered to have cellular DNA damage. The small division number permits potential mutagens to act on replication DNA. Reproducibility is a prerequisite to consider a compound as positive in this test. Results are dependent on the differential inhibition of the H17 and M45 strains by DNA modifying agents. This effect is expressed as differences in the size of the zone of growth inhibition. The combined incubation of the compound and the cells permits constant exposure for 24 hr.

The study is accepted.

B.6.4.2b *In vivo* genotoxicity testing (somatic cells) (Annex IIA 5.4.2)**B.6.4.2.1b *In vivo* micronucleus test (Annex IIA 5.4.2)****- Technical methylisothiocyanate (MITC): Mouse micronucleus test (Allen et al, 1985)**Findings:

A preliminary toxicity test indicated that a dosage of 110 mg/kg would be expected to kill approximately 10% of the animals within 72 h of dosing (table B.6.4.2.1-1).

Results of the test with MITC at the 24, 48 and 72 hr sampling times are presented in table B.6.4.2.1-2. One female died after 110 mg MITC/kg. The incidence of micronucleated polychromatic and normochromatic erythrocytes was comparable with controls at all three sampling times while mitomycin gave the expected results. At the 48 hr, sampling time there was a reduction in the poly/normo ratio in MITC treated mice indicating bone marrow cell toxicity.

Table B.6.4.2.1-1b: preliminary toxicity test- mortality data.

Dose (mg/kg)	Mortality	
	M	F
Phase I		
Vehicle corn oil	0/2	0/2
20	0/2	0/2
40	0/2	0/2
80	0/2	0/2
160	2/2	2/2
320	2/2	2/2
Phase II		
Vehicle control	0/5	0/5
80	0/5	0/5
100	0/5	0/5
125	0/5	2/5
156.25	5/5	5/5

Table B.6.4.2.1-2b: results of micronucleus test

Sampling time; Test substance dose (mg/kg)	Mean ratio poly/normo erythrocytes	Mean n° micronucleated polychromatic erythrocytes ‰	Total n° micronucleated normochromatic erythrocytes ‰
24 hr			
Vehicle control	0.961	0.7	0
MITC 110 mg/kg	0.766	0.7	0.9
Mitomycin 4 mg/kg	0.410*	47.5*	0.9
48 hr			
Vehicle control	1.012	0.5	0
MITC 110 mg/kg	0.579*	0.7	0.3
72 hr			
Vehicle control	1.159	0.3	0.2
MITC 110 mg/kg	1.020*	0.4	0.2

* Statistically significantly different from control.

Conclusion:

MITC showed no evidence of mutagenic potential when administered in corn oil to mice, by gavage, in this *in vivo* test.

GLP status: attest of study director.

Guidelines: experimental protocol not fully in agreement with 2000/32/CE Annex 4C, 92/69-84/449/CE, or OCDE 474 (1997-83)

Deviation from official protocol: peripheral red blood cells were not analyzed; micronuclei were examined on 1000 PCE (in agreement with 84/449). Criteria for accepting the test are not reported.

Material and methods: CD mice received by gavage MITC (batch n° 340178 B19 for preliminary test and batch n° 340173 B23 for the main test at 95.86%) diluted in corn oil.

The preliminary test was performed in 2 phases with 2 mice/sex /dose at 20, 40, 80, 160, or 320 mg/kg bw and 5 mice/sex/dose received MITC at 80, 100, 125 or 156.25 mg/kg. Following dosing, animals were observed for a 72 h period.

The main test was performed at 110 mg/kg bw with 15 mice/sex. Groups were killed 24, 48 and 72 hr after dosing. Femurs were used for bone marrow smear. 4 mice/sex received mitomycin as positive control at 4 mg/kg bw.

The study is accepted.

Data from the open literature:

- **Genotoxic effects of methyl isothiocyanate (Kassie et al, 2001)**

According to the authors of the study, MITC caused only marginal mutation induction in reversion assays with *Salmonella* strains TA100 and TA98 and, the maximum effect (<2-fold increase over the background rate) was seen at 100 µg/ml.

In differential DNA-repair assays with *E. coli* (strains 343/763 *uvrB/recA* and 343/765 *uvr⁺/rec⁺*), a pronounced dose-response effect (induction of repairable DNA-damage) was seen at low concentrations (≥ 4 $\mu\text{g/ml}$). In both bacterial assays, addition of activation mix (rat liver S-9) led to a reduction of the genotoxic effects.

In micronucleus assay and in single cell gel electrophoresis assay with human hepatoma cells (HepG2), clear cut positive results were obtained at exposure concentrations of < 5 $\mu\text{g/ml}$.

On the contrary, only marginal effects were seen in differential DNA-repair host-mediated assays where *E. coli* indicator cells were recovered from different inner organs of mice that had been treated orally with a high dose (90 mg/kg bw) of the test compound. Further *in vitro* experiments showed that MITC is inactivated by body fluids (saliva, gastric juice) and that its DNA-damaging properties are attenuated by non-enzymatic protein binding. Since exposure of HepG2 cells to MITC led to formation of thiobarbituric acid reactive substances, it is likely that its DNA-damaging effects involve lipid peroxidation processes. Overall, the findings show that MITC induces only marginal effects at extremely high (almost lethal) doses in inner organs *in vivo*, but it causes DNA-damage at low concentrations *in vitro*.

Comment from RMS: these studies, although not performed according to the current guidelines or with protocols not included in the current guidelines confirm the negative results reported in the studies provided by the company.

Material and methods:

MITC was from Sigma, liver S9 was from Arochlor pretreated rats.

Salmonella typhimurium strain TA 98 and 100 were used with MITC at 0-200 $\mu\text{g/plate}$ in the plate incorporation assay or according to the preincubation assay with or without S9.

DNA repair assay with *E. coli* strains repair deficient 343/753 (*uvr/rec*) *Lac⁺* and repair proficient 343/765 (*uvr⁺/rec⁺*) *Lac⁻* were used in liquid incubation experiments and in animal mediated assays performed according to a publication (?). MITC (90 or 270 mg/kg) was given by gavage to 3 male mice that received an iv injection of concentrated strains and animals were killed 2 hours later. An *in vitro* test was performed with *E. coli* liquid incubation, 0-25 $\mu\text{g/mL} \pm \text{S9}$.

Micronucleus test and Single cell gel electrophoresis were performed with Hep G2 cells. In the Human Hep G2, Single cell gel assay, MITC was at 0, 3.65, 5.43 $\mu\text{g/mL}$ and (IV) micronucleus *in vitro*, 0.5-4 $\mu\text{g/mL}$.

The study is accepted as additional information.

B.6.4.3 In vivo studies in germ cells (Annex IIA 5.4.3)

An *in vivo* study in germ cells is only justified when any result of an *in vivo* study in somatic cells is positive. The results of the *in vivo* studies demonstrated that Metam-sodium or MITC has no mutagenic or genotoxic potential *in vivo*. Therefore, there was no necessity to evaluate Metam-sodium or MITC in an *in vivo* study using germ cells.

B.6.4.4 Summary of genotoxicity (Annex IIA 5.4)**A) Studies with Metam-sodium**

Metam-sodium was evaluated for possible mutagenic/genotoxic effects in *in vitro* test systems using bacterial and mammalian cells and in *in vivo* test systems using hamsters and mice.

In the bacterial revertant assay, employing the standard plate test as well as the preincubation test method Metam-sodium was negative in the presence or absence of metabolic activation towards *S. typhimurium* strains TA 92, TA 98, TA 100, TA 1535, TA 1537 and TA 1538.

In the HGPRT assay in CHO cells, slight increases in mutation rates cannot be ruled out completely although the results are not dose-related.

Metam-sodium revealed a slight clastogenic activity in the presence and absence of metabolic activation in the first chromosome aberrations test and in the presence of metabolic activation system in the second test, both performed in human lymphocytes. Increases in structural chromosomal aberrations were observed at concentrations that had cytotoxic effects on chromosomal morphology which prevented metaphases from accurate analysis for structural chromosomal aberrations.

In the *in vitro* UDS assay to assess DNA damage in freshly isolated primary rat hepatocytes metam-sodium did not induce unscheduled DNA synthesis even at concentration being cytotoxic as demonstrated by the net nuclear grain count and the percentage of nuclei with more than 6 or 20 grains.

Metam-sodium did not induce micronuclei in bone marrow polychromatic erythrocytes in male and female CD-1 mice and does not possess clastogenic activity in the mouse bone marrow micronucleus test.

In the *in vivo* chromosome aberration test performed in Chinese hamsters, Metam-sodium is not clastogenic.

In conclusion, Metam-sodium induced equivocal results in *in vitro* tests evaluating mammalian gene mutations and chromosomal aberrations.

Metam sodium did not induce unscheduled DNA synthesis in primary hepatocytes. *In vivo* cytogenic investigations were negative. Overall, it can be concluded that metam sodium is not genotoxic *in vivo*.

Table B.6.4.4-1: Summary of genotoxicity of Metam

Type of test Cell/Test species	Conditions	Test substance purity	Results	References
<i>In vitro</i> tests:				
<i>Salmonella typhimurium</i> strains TA 92, TA 98, TA 100, TA 1535, TA 1537 and TA 1538	Standard plate test: 20 – 5,000 µg/plate (+ S9/-S9) Preincubation test: 4 – 5,000 µg/plate (+ S9/-S9)	Batch n° ZH130585; 42.2%	Negative	Engelhardt, (1987)
CHO cells (HGPRT) locus	0.0000464 - 0.01mg/mL (+/-S9)	Batch n° ZH130585; 42.2%	Equivocal	Engelhardt, (1987)
Chromosomal aberrations in human lymphocytes	+ S9: 10 – 40 µg/mL - S9: 1 – 20 µg/mL	Batch n° ZH130585; 42.2%	Positive	Gelbke and Engelhardt, (1987)
Chromosomal aberrations in human lymphocytes	+ S9: 5 – 40 µg/mL - S9: 2.5 – 30 µg/mL	Batch n° WCR 14989-13- 01 ; 32.3%	Equivocal	Mackay, (1996)
UDS in hepatocytes	primary rat hepatocytes 0.5 – 250 nL/mL	Batch n° ZH130585; 42.2%	Negative	Cifone, (1987)

Type of test Cell/Test species	Conditions	Test substance purity	Results	References
In vivo tests				
Micronucleus test - Bone marrow cells of CD1-mice	single oral dose males and females: 500 mg/kg bw bone marrow sampling 24 and 48 h after dosing	Batch n° WCR 14989-13- 01 ; 32.3%	Negative	Barber and Mackay (1996)
CA in bone marrow cells of Chinese hamsters	single oral dose (gavage) males and females: 0, 150, 300 and 600 mg/kg bw bone marrow sampling 6, 24 and 48 h after dosing	Batch n° ZH130585; 42.2%	Negative	Gelbke and Engelhardt (1987)

B) Studies with MITC

MITC was not mutagenic in *Salmonella thyphimurium* strains TA98, TA100, TA1535, and TA1537 in the presence and absence of metabolic activation.

MITC has no chromosomal damaging effects in an *in vitro* test using human lymphocytes.

MITC was further negative in recombination assays in *Bacillus subtilis* strains H17 and M45 with and without metabolic activation.

In the *in vivo* mouse micronucleus test, MITC did not increase the number of micronucleated polychromatic erythrocytes.

MITC is not genotoxic. From open literature, it was reported that MITC was not genotoxic.

Table B.6.4.4-2: Summary of genotoxicity of MITC.

Type of test Cell/Test species	Conditions	Test substance purity	Results	References
In vitro tests				
<i>Salmonella</i> <i>typhimurium</i> strains TA98, TA100, TA1535 and TA1537	+ S9: 20 – 5,000 µg/plate - S9: 20 – 5,000 µg/plate	Batch n° 6205 MK. 98.1%	Negative	Engelhardt, and Gelbke, 1986
Recombination assays with <i>Bacillus subtilis</i> H17 (rec ⁺) and M45 (rec ⁻)	+ S9: 1 – 10,000 µg/plate - S9: 1 – 10,000 µg/plate	Id n° 85/231 charge 6205 MK; 98.1%?	Negative	Jagannath, 1989
CA in human lymphocytes	+ S9: 0.05, 0.1, 0.5 µg/mL - S9: 0.1, 0.5, 1.0 µg/mL	Batch n° 6205 MK. 98.1%	Negative	Engelhardt and Gelbke, 1987
In vivo tests				
Micronucleus test in bone marrow of CD1 Swiss mice	single oral dose males and females: 110 mg/kg bw bone marrow sampling 24, 48 and 72 h after dosing	Batch n° 340178 B19 and batch n° 340173 B23 at 95.86%	Negative	Allen et al (1985)

B.6.5a Long-term toxicity and carcinogenicity of metam sodium (Annex IIA 5.5)

B.6.5.1a Long-term (2 years) oral toxicity in the rat (Annex IIA 5.5)

- Metam Sodium: Two year drinking water study in rats (Rattray, 1994)

Findings:

At 0.19 mg/ml water was not drinkable and this dose level was established as the MTD.

Mortality: The high incidence of treatment-related chronic progressive glomerulonephropathy contributed to the early termination of several males.

Clinical signs: male rats at top dose had reduced hindlimb function and were hunched and tin.

Body weight: was reduced throughout the study in all rats at top dose. In females at 0.056 mg/ml, there was a less marked reduction.

Water consumption: was reduced in all treated groups throughout the study with the effect being more marked in females. Males of all groups showed a gradual increase in water consumption from week 52 until week 96 but the differential consumption between the groups was maintained.

Food consumption: was reduced at top dose in all rats and in females at 0.056 mg/ml throughout the study. At top dose, food utilization was slightly less than in controls during the first 12 weeks of the study.

Ophthalmoscopy: no evidence of relationship to treatment.

Hematology: slight altered parameters, both in interim and terminal kill at 0.056 and 0.19 mg/ml together with the increased hemosiderin deposition seen in spleen in animals at top dose may be indicative of a mild hemolytic anemia. These changes are considered compound related.

Clinical chemistry: in blood, there was a slight reduction in ALT and AST in females at top dose and in ALT in females at 0.056mg/ml throughout the study reaching sometimes-statistical significance. Plasma phosphorus levels were reduced in females. These effects are considered not to be of toxicological importance.

Urinary volume was slightly lower at top dose and intermediate dose with a concomitant increase in specific gravity at top dose. Slight acidosis was seen in the first six months of the study in males at top dose.

Organ weight: relative kidney weight was increased in females at top dose and absolute kidney weight was decreased in males at interim kill.

Macroscopic findings: A wide variety of changes but none was of clear treatment related.

Microscopic findings:

Effects on the nasal passage were largely confined to top dose animals although two males given 0.056 mg/ml also showed minimal changes. The lesion affected primarily the Bowman's glands and ducts but in some animals, there was also degeneration of the olfactory epithelium. The lesion was indicative of a systemic effect both because of its location in the posterior part of the nasal passages and because Bowman's and Steno's glands (the lateral nasal gland located lateroventrally to the maxillary sinus) are known to be metabolically active. The incidence of rhinitis was slightly increased in males at top dose but was not considered to be related to treatment. The rhinitis could have exacerbated the changes in Bowman's glands, but there were also several rats with moderate hypertrophy of Bowman's glands/ducts without rhinitis.

The reduced hindlimb function seen in males given 0.19 mg/ml metam correlated with the pathological findings of degenerative myopathy of voluntary muscle. This pathological finding was present in all groups and is common in ageing rats but although the incidence was similar in all groups, including controls, there was an increase in the severity of myopathy in rats given 0.19 mg/ml.

In spleen, there was a slight increase of hemosiderin deposition in both sexes at top dose.

Males had vascular ectasy in adrenals at top dose. This is a common change in old rats of both sexes, with a higher incidence in females. The significance of this increase is unclear.

Spontaneous, chronic progressive glomerulonephropathy were reported and correlated with the macroscopic changes described in the kidney (pale and enlarged kidneys). The severity of the lesion was similar in all groups.

Neoplastic findings: there were no significant neoplastic findings in interim kill animals and there were no treatment-related neoplasm in intercurrent or terminal rats.

There was an increase over controls in the number of males with malignant tumors at 0.056 and 0.19 mg/ml and in males given 0.056 mg/ml with multiple tumors. The number of males given 0.056 mg/ml with malignant tumors was greater than that seen in males at top dose. Of the eight liver masses recorded macroscopically in males at top dose, four were hepatocellular adenocarcinoma, three were hepatocellular adenoma and one was a

hepatoblastoma. The incidence was similar to that of the controls. Hemangiosarcoma incidence in mesenteric lymph node in males at 0.056 mg/ml was slightly higher than the control. This effect accounts probably for the overall increase in male rats with malignant tumors at this dose level.

Table B.6.5.1-1a: long-term (2 years) oral toxicity in the rat.

Dose/endpoints	0		0.019		0.056		0.19 mg/mL	
	M	F	M	F	M	F	M	F
Mean dose mg/kg bw/d:	0	0	1.5	2.7	4.3	6.8	12.5	16.8
Mortality :	42	28	37	28	33	29	36	23
Body weight:								
Week 4						↓2%*	↓6%*	↓5%*
Week 13						↓3%*	↓7%*	↓7%*
Week 51						↓5%*	↓8%*	↓11%*
Week 104-105							↓12%*	↓12%*
Water consumption:								
Week 4					↓16%*	↓23%*	↓43%*	↓56%*
Week 13				↓11%*		↓32%*	↓34%*	↓16%*
Week 51-52				↓12%*		↓24%*	↓20%*	↓47%*
Week 104-105							↓22%*	↓29%*
Food consumption								
Week 4						↓7%*	↓8%*	↓11%*
Week 13			↓3%*			↓6%*	↓10%*	↓8%*
Week 51-52						↓8%*	↓5%*	↓15%*
Week 104-105							↓11%*	
Food utilization								
Week 1-12							↓2%*	↓7%*
Hematology:								
Hb								
Week 14							↓3%*	
Week 53							↓3%*	
Week 105					↓17%*		↓12%*	↓5.1%*
Ht								
Week 105					↓15%*		↓10%*	↓4%*
RBC count								
Week 105					↓16%*		↓10%*	↓8%*
MCV Week 105							↑17%*	
MCH Week 105								↑3.5%*
Monocytes count week 105							↓66%*	
Clinical chemistry plasma:								
ALT								
Week 14								↓15%*
Week 27						↓25%*		↓27%*
AST								
Week 14								↓11%*
Total protein : week 52						↓4%*		↓5%*
Creatine kinase week 52						↓25%		↓37%*
Phosphorus week 52								↓10%*
Sodium								
Week 79								↓12%*
Week 105			↑2%*		↑2.3%*		↑1.7%*	
Glucose week 105						↑16%*		↑14%*
triglycerides week 105						↓25%*		↓43%*
Urinalysis								
PH								
Week 13	6.58		6.5		6.47		6.31*	

Dose/endpoints	0		0.019		0.056		0.19 mg/mL	
	M	F	M	F	M	F	M	F
Week 26	6.56		6.44		6.49		6.33*	
Proteins :								
Week 26							↓30%*	
Wk 52						↓80%*	↓38%*	↓70%*
Wk 78						↓77%*	↓34%*	↓70%*
Volume								
Week 52						↓32%*	↓26%*	↓30%*
Week 78								↓29%*
Specific gravity								
Week 52								↑0.4%*/
Week 78								↑0.5%*
Urinary qualitative tests : week 104								
Glucose	16		18		23		25	
ketones	13		14		19		21	
Urobilinogen	16		18		23		25	
Organ weight:								
Kidney (a)							↓12%*	
Kidney (r)								↑17%*
Microscopic findings:	Number affected animals							
Total: n° examined rats	64	64	64	64	64	64	64	64
Adrenal gland								
vascular ecstasy total	4	54	6	48	7	50	12	44
Harderian gland :								
mononuclear cell infiltration	8	11	6	12	9	12	12	11
Kidney chronic progressive glomerulonephropathy	64	47	62	39	63	36	61	38
Liver spongiosis/peliosis hepatic with altered hepatocytes total	7	1	5	0	15	3	13	2
Nasal cavity:								
Rhinitis total	1	1	4	3	2	4	9	4
Bowman's gland /duct hypertrophy total	0	0	0	0	2	0	39	43
Olfactory epithelium								
hyperplasia total	0	0	0	0	0	0	7	2
degeneration total	1	0	0	0	0	1	28	5
Steno's gland atrophy total	11	13	12	14	15	17	49	43
adenitis total	14	1	4	1	13	10	23	9
Respiratory epithelium hyperplasia total	0	0	0	1	0	0	4	2
Sciatic nerve degeneration total	47	37	42	34	45	36	47	39
Spleen increased haemosiderin slight	2	10	2	6	2	8	8	20
Increased porphyrin slight	6	9	8	3	14	8	18	5
Uterus glandular dilatation total		2		6		8		8
Voluntary muscle degenerative myopathy total	37	24	39	17	36	15	44	27
Neoplastic findings:								
Liver hepatocellular adenocarcinoma	3	0	2	0	4	2	5	0
Hepatoblastoma	0	0	0	0	0	0	1	0
Mesenteric lymph node hemangiosarcoma	0	0	2	3	5	1	2	0
hemangioma	9	2	3	1	3	3	8	0

Dose/endpoints	0		0.019		0.056		0.19 mg/mL	
	M	F	M	F	M	F	M	F
Sum	9	2	5	4	8	4	10	0
Brain meningioma (benign)	0	1	1	0	3	1	1	1
Overall tumor incidence:								
Number tumor bearing rats	45	56	39	55	48	53	51	51
Rats with malignant tumors	5	11	9	11	16	11	13	10
Rats with benign tumors	43	53	38	53	46	53	49	49
Rats with metastasis tumors	0	3	3	4	1	5	4	5

Conclusion:

NOAEL = 0.019 mg/ml corresponding to 1.5 mg/kg bw/d based on the effects on the nasal passages, body weight effects, changes in some hematological parameters seen at 0.056 mg/ml. There was a slight increased incidence of mesenteric lymph node hemangiosarcoma in male rat at 0.056 mg/ml. Such an effect was not seen at top dose. There was no indication of an early onset of tumors associated with the administration of metam. In view of the overall results obtained in this carcinogenicity study, a carcinogenic potential of metam sodium cannot be identified.

From the open literature: According to a Memorandum Office of Environmental Health Hazard Assessment (2003) of Metam sodium qualitative risk assessment based on Hsd/Ola: Wistar tox rat and C57BL/10JfCD-1/Alpk mouse drinking water studies:

The interpretation of the rat data on tumor incidence was ambiguous with respect to hemangiosarcoma induction. Although there was a statistically significant increase in hemangiosarcoma incidence in the mid dose relative to controls and all metam treated groups showed a greater incidence than the control group, a clear dose-response relationship was not observed. Further, historical control data are not available [for this strain?] making interpretation of the mid dose increase in incidence difficult. When incidences of hemangiomas and hemangiosarcomas were combined as suggested by the NTP, no increase with dose was evident. The incidence of another vascular tumor type, benign meningiomas, did, on the other hand, exhibit biphasic behavior, though the low absolute rates precluded a determination that they were treatment related. Without historical controls, the absence of hemangiosarcomas and meningiomas in the concurrent controls must be accepted as representative of their historical behavior.

GLP status: yes (no attest of competent authority)

Guidelines : experimental protocol not fully in agreement with Directive 87/302/CEE Annex V B or OECD test guideline 453 (1981).

Deviation from official protocol: limited number of animals: 52 (2 year) + 12 (1 year) rats/sex/dose and 3 doses, identical for chronic toxicity and carcinogenicity. Ornithine decarboxylase was not measured. Weight of (Para) thyroid (OK for OECD) and ovaries are missing. Raw data of clinical signs are not reported.

Material and methods: 64 Hsd/Ola: Wistar-tox (closely related to Alpk:APfSD) rat/sex/dose received in their drinking water metam sodium (batch n° BAS/005/OON 90-2; 525.54 g/L = 43.148% w/w) at 0, 0.019, 0.056 or 0.19 mg/ml corresponding to 1.5, 4.3, 12.5 mg/kg bw/d for males and 2.7, 6.8 and 16.8 mg/kg bw/d for females for up to 104 weeks. 12 rats from each group/sex were killed after 52 weeks. Drinking water was prepared daily and analyzed twice weekly for the first 2 weeks, once a week for the next 2 weeks and then one batch were analyzed. pH of drinking water was adjusted to 8-9. The drinking water formulations were analyzed at intervals throughout the study. The achieved concentrations were generally within 10% of the nominal concentrations and the overall stability of the formulations was generally within 12% of the nominal concentration after 24 h. Based on nominal drinking water levels dose rates were calculated in mg/kg bw/d.

Statistics: BW analysis of covariance. Food, water consumption and utilization, hematology and clinical and urine chemistry, and organ weight were analyzed by variance. Differences from control were based on Student T test. Kaplan-Meier survival estimates were calculated separately for sex and treatment group. Log rank test was used. Incidence of tumors was examined by the Cochran Armitage test. The study is accepted.

B.6.5.2a Carcinogenicity study in the rat (Annex IIA 5.5)

See point B.6.5.1a.

B.6.5.3a Carcinogenicity study in the mouse (Annex IIA 5.5)**2 year drinking study in mice (Horner, 1994)**Findings:

Mortality: no effects.

Clinical signs: no treatment-related effects.

A transitional cell papilloma of the urinary bladder was present in one male and one female top dose. Whilst this tumor type is a relatively common neoplasm in this strain of mouse, the increased incidence in top dose mice is significant and suggests an association with treatment. The small increase in angiosarcoma of the subcutaneous tissue correlated with the increase in macroscopic masses in males and females at top dose (table B.6.5.3-2a).

Angiosarcoma were seen in several organs (spleen, liver, subcutaneous tissue and bone marrow). It was not certain whether the tumors represented primary tumors with metastasis deposits or multicentre tumors; therefore, it was argued that the designation of tumors into primary sites was inappropriate but the treatment-related increase in angiosarcoma, in a variety of sites, was a factor contributory to death at top dose.

Consequently, as shown in table B.6.5.3-2a, the overall incidence in mice with angiosarcoma in any site was calculated. For angiosarcoma in any site, there was no treatment-related change in tumor latency but the increased incidence of angiosarcoma in top dose animals is regarded as significant when compared with data from concurrent and historical control data. In contrast, the incidence of angiosarcoma at low and mid dose levels is consistent with the historical control data and is considered to be of no toxicological significance.

With respect to the spontaneous incidence of angiosarcoma, historical background data from 9 studies starting from March 1984 to February 1991 were provided including angiosarcoma at all sites in the strain of mice in this study. This historical incidence data is summarized in table B.6.5.3-3a.

It is clear that at the top dose level the incidences are both statistically and biologically higher than could be accounted for by a random event. Hence, a test substance related effect must be assumed at top dose level. It is also obvious that the increase in angiosarcoma is much higher in males than in females. In this context, it is of interest to note that the spontaneous incidence of this neoplastic lesion is also much higher in males than in females, as evidenced by the historical control data.

Table B.6.5.3-1a: 2-year metam drinking water study in mice.

Endpoints/dose	0		0.019		0.074		0.23 mg/ml	
	M	F	M	F	M	F	M	F
Mean received dose (mg/kg bw/d)	0		1.9	2.6	7.2	9.6	28.9	31.2
Body weight wk 105							↓7%*	↓2%
Bw gain							↓16%	↓6%
Food consumption					↓ ± 5%* wk 24-52		↓ ± 5%* wk 2-64	
Water consumption					↓ 8-12%* wk 1-78	↓ 10-16%* wk 3-11	↓ 15-6%* wk 1-2	↓ 12-18%* wk 1-12
							↑ 10-24%* wk 9-104	↑ 10%* wk 78-104
Hematology wk 105								
RBC count								↓ 10%*
Mean cell volume			↓ 4 %*		↓ 4%*			↑ 5 %*
Mean cell hemoglobin							↑ 3 %*	↑ 5 %*
Organ weight:								
Liver rel					(↑10 %)	↑ 16%*	↑ 44 %*	↑ 23 %*
Kidneys rel								↑ 12 %*
Epididymides rel							↓ 23%*	
Macroscopic findings:								
Multiple masses	6	1	1	3	4	3	9	4
Subcutaneous tissue: mass	2	1	2	3	2	1	5	5
Liver pale	7	6	5	5	6	6	11	9
Urinary bladder: distended	6	1	4	0	7	1	9	1
Seminal vesicle enlarged	16		7		7		1	
Microscopic findings in all mice:								
Spleen:								
extramed.hematopoiesis total	11/55	16/55	19/55	19/55	12/55	19/55	21/55	25/55

Endpoints/dose	0		0.019		0.074		0.23 mg/ml	
	M	F	M	F	M	F	M	F
Mean received dose (mg/kg bw/d)	0		1.9	2.6	7.2	9.6	28.9	31.2
Urinary bladder:								
epithelial hyperplasia total	0/54	0/54	0/55	0/52	2/55	10/53	45/55	44/55
Sub mucosal inflammatory cell infiltr. total	0	0	0	0	0	0	4/55	9/55
Submucosal hyalinization total	0	0	0	0	0	1/53	45/55	26/55
Mononuclear cell infiltration total	8/54	34/54	7/55	25/52	5/55	21/53	32/55	29/55
Eosinophilic cytoplasmic inclusion epithelium total	0	5/54	5/55	42/52	50/55	1/53	33/55	47/55
Increased submucosal connective tissue total	1/54	0	0	0	0	2/53	11/55	19/55
Liver fat vacuolation	4/55	1/55	2/55	0/55	5/55	1/55	20/55	6/55
Areas hepatocyte necrosis		1/55			1/55	3/55	0/55	4/55
Adrenals:								
Cortical ceroidosis total	42/54	25/55	37/52	15/55	37/55	11/54	11/54	14/55
Epididymis mononuclear cell infiltration total	3/55		0/52		1/54		7/55	
Testis atrophy moderate	0/55		3/54		3/54		5/55	

Table B.6.5.3-2a: Intergroup comparison of microscopic neoplastic findings (malignant)

Endpoints/dose	0		0.019		0.074		0.23 mg/ml	
	M	F	M	F	M	F	M	F
Mean received dose (mg/kg bw/d)	0		1.9	2.6	7.2	9.6	28.9	31.2
Overall incidence of mice with angiosarcoma in any site:								
	7/55	4/55	12/55	2/55	12/55	6/55	27/55	10/55
Intercurrent group: n° affected tissue/n° examined								
Spleen angiosarcoma :	4/34	0/27	2/35	1/30	6/28	2/36	12/35	2/33
Liver: angiosarcoma	0/34	0/27	2/35	0/30	2/28	0/36	3/35	1/33
%	11.8	0	5.7	3.3	21.4	5.6	34.3	6.1
Lymphoreticular tissue : composite lymphoma (malignant)	23/27	19/22	23/28	21/26	14/17	20/26	19/19	19/24
Subcutaneous tissue angiosarcoma	0/2	0/2	0/2	1/3	1/4	1/2	1/3	3/4
Terminal mice : n° affected tissue/n° examined								
Spleen angiosarcoma	2/21	0/28	0/20	0/25	2/27	1/19	3/20	2/22
Liver: angiosarcoma	0/21	0/28	4/20	0/25	1/27	1/19	4/20	2/22
Subcutaneous tissue angiosarcoma :	1/4	0/2	1/3	0/1	0/0	0/19	2/4	0/1
Total mice : n° affected tissue/n° examined								
Spleen angiosarcoma :	5/55	0/55	2/55	1/55	8/55	3/55	15/55	4/55
Liver: angiosarcoma	0/55	0/55	6/55	0	3/55	1/55	7/55	3/55
Subcutaneous tissue angiosarcoma	1/6	0/55	1/5	1/4	1/4	1/4	3/7	3/5
fibrosarcoma	0/55	1/55	0	0	0	0	0	0
Bone marrow	0/55	0/55	2/54	0	0	0	0	0
Lymph nodes mesenteric angiosarcoma	0/55	0/55	1/55	0	0	0	0	0

Endpoints/dose	0		0.019		0.074		0.23 mg/ml	
	M	F	M	F	M	F	M	F
Mesentery angiosarcoma	0/55	0/55	0	0	0	0	1/55	0
Thoracic angiosarcoma	1/55	0/55	0	0	0	0	0	0
Ovary angiosarcoma		2/55	0	0	0	0	0	0
uterus angiosarcoma	0/55	0/55	0	0	0	1/55	0	0
Limb angiosarcoma	0/55	1/55	0	0	0	0	1/55	0
sternum angiosarcoma	0/55	1/55	0	0	0	0	0	0
Overall incidence of mice with angiosarcoma in any site								
N° mice	7/55	4/55	12/55	2/55	12/55	6/55	27/55	10/55
%	12.7	7.3	21.8	3.6	21.8	10.9	49	18.2
Urinary bladder:								
Transitional cell carcinoma malignant	0	0	0	0	0	0	0	1/55
Transitional cell papilloma benign	0	0	0	0	0	0	1/55	0

Table B.6.5.3-3a: Historical incidence data for angiosarcoma in C57 BL mice

	Males	Females
Range	5 - 18.33%	0 - 9%
Mean of means $\pm$ standard deviation	10.08% $\pm$ 4.37%	4.77% $\pm$ 3.07%

Conclusion:

Transitional cell papilloma/carcinoma affected two mice at top dose. Both male and female mice had a dose-related increase in non-neoplastic urinary bladder lesions. The pathological significant changes were mainly present at 0.23 mg/ml with a much-reduced incidence at 0.074 mg/ml, although eosinophilic inclusion bodies were present in increased amounts in the epithelial cells of all treated groups.

At 0.074 and 0.23mg/ml, there was an increased incidence of angiosarcoma in males and females.

Metam sodium is carcinogenic in mice.

NOAEL systemic toxicity and carcinogenicity = 0.019mg/ml (1.9 mg/kg bw/d) taking into account the increased incidence of urinary bladder epithelial hyperplasia, hepatocytic necrosis and increased incidence of angiosarcoma at 0.074 mg/ml (7.2 mg/kg bw/d).

GLP status: yes (no attest of competent authority)

Guidelines: not fully in agreement with Directive 87/302/CEE Annex V B or OECD test guideline 451 (1981).

Deviation from official protocol: Hematology tests were performed on 10 animals/sex for control and top dose group only instead of blood smears on all animals of these groups.

Material and methods: 55 C57 BL/10JfCD-1/Alpk mice/sex/dose received in drinking water metam sodium (batch n° BAS/005/00N 90*2; 525.54 g/L (43.148%)) at 0, 0.019, 0.074 or 0.23 mg/ml for a period of 2 years. Drinking water was at pH 8-9 and was prepared daily. The achieved dose levels were generally within 10% of the nominal concentration. The stability of metam sodium in drinking water was determined over 24 hr. A mean reduction of approximately 7-11% from the initial analysis values was seen over 24 hr at all drinking water concentrations examined. The dose received fell markedly during the initial phase of the study corresponding to the rapid growth phase of the mice. The mean dose received values for males receiving 0.019, 0.074 and 0.23 mg/ml were 1.9, 7.2 and 28.9 mg/kg bw/d for males and 2.6, 9.6 and 31.2 mg/kg bw/d for females.

Ten males and females were used as microbiological sentinels and assigned to group 0.23 mg/ml and were checked daily to provide information about the microbiological status of experimental mice during the study.

Statistics: bw and organ weight was analyzed by covariance; food and water consumption, hematology was considered by analysis of variance. All statistical tests were two-sided. Fisher's exact test was used as well as Cochran Armitage test.

The study is accepted.

Additional comment from the open literature: According to a Memorandum Office of Environmental Health Hazard Assessment (2003) of Metam sodium qualitative risk assessment based on Hsd/Ola: Wistar tox rat and C57BL/10JfCD-1/Alpk mouse drinking water studies:

"The increase in angiosarcoma incidence in mice at all sites was highly significant at the high dose compared to controls ($p < 0.001$, Fisher Exact Test). Likewise, the increases in angiosarcoma incidence (i.e., liver, spleen, and bone marrow) were significant for angiosarcoma. The dose-response curve was also positive for trend ($p < 0.001$, Cochran-Armitage trend test). While Fisher Exact tests in females were not significant at any dose ($p > 0.05$) when angiosarcomas at all sites were evaluated, the increase in incidence of this tumor in the spleen was

Carcinogenic potential of metam in the 2 year study in mice (Horner 1994) was during Peer Review

RMS: It was considered that the increased incidence of angiosarcoma reported at mid dose level (0.074 mg/mL) should be taken into account for setting of carcinogenicity NOAEL. The allocation of this carcinogenicity NOAEL was triggered by the increased incidence in spleen angiosarcoma at all but the lowest doses in terminal mice (5/2/8/15), while the incidence at any site was 7/12/12/27. It is debatable whether murine angiosarcoma findings, which are often (but not always) multicentric in origin, preclude the consideration of their occurrence in one specific organ. In the case of Metam, most angiosarcomas were observed in the spleen, and in the absence of HCD in this organ, they should be taken into account by precaution.

When the incidences in all animals ($\text{♂}+\text{♀}$) were considered, both the rises in the spleen (5,3,11,19) and at any site (11,14,18,37) were indicative of the carcinogenic potential of Metam-Na in the mouse.

During Peer Review, it was concluded that the lowest dose used was a NOAEL rather than a LOAEL, taking account that the findings in the liver showed no dose response. It was concluded that classification Carc. Cat. 3 rather than Carc. Cat. 2 was justified based on the effects seen in the spleen only.

B.6.5b Long-term toxicity and carcinogenicity of MITC (Annex IIA 5.5)

B.6.5.1b Long-term (2 years) oral toxicity in the rat (Annex IIA 5.5)

- **T27/2 Methyl isothiocyanate: a chronic oral (drinking water) toxicity and carcinogenicity study in the rat (Brown, 1984, 1988)**

Findings: table B.6.5.1-1b.

Survival rate was not adversely influenced by treatment.

Clinical signs: neither the incidence nor the severity of changes appeared to be affected by treatment.

Body weight: all male rats of treated groups gained less weight than controls throughout the study affecting the terminal body weight. Dose-effect relationship was not evident.

Food consumption was not affected

Water consumption: treated rats tended to drink less than controls, particularly the males. Although this slight reduction in water intake was not directly related to MITC concentration, the effect appeared to be most marked in those rats receiving the highest dose.

According to the company, although female body weight gains did not appear to be affected in the mid and high dose groups, water consumption was decreased although not as consistently as the males. This strongly suggests that higher dose levels offered to females may lead to palatability problems and thus compromise the study.

Ophthalmoscopy: no compound related effects were seen.

Hematology: transient changes in some white blood cell parameters were reported essentially at top dose without reaching statistical significance.

Clinical chemistry: no compound related changes.

Urinalysis: blood traces in urine of high dose males was not associated with an increased excretion of epithelial cells or casts.

Organ weight: slight changes were seen in the absolute and relative weights of certain organs. These changes could reflect the pattern of body weight differences. They are, therefore, a secondary effect of treatment and considered to be of no toxicological significance as similar changes are seen in undernourished rats.

At necropsy, at top dose, a slight increase in skin subcutis masses, and pituitary enlargement were seen.

histopathology (reported in table B.6.5.1-2b): In rats dying during the first 52 weeks of study, no significant intergroup differences were observed from the data to suggest a relationship to exposure of MITC.

Common findings were a higher incidence of Harderian gland adenitis, kidney microcalculi, liver cell vacuolation, biliary proliferation, and spleen hyperplasia and ovary cysts. Some of these findings occurred at a slightly higher incidence at top dose.

Table B.6.5.1-1b: 101-week exposure of rats to MITC.

Endpoints/dose	0		2 ppm		10 ppm		50 ppm	
	M	F	M	F	M	F	M	F
Calculated dose mg/kg bw/d			0.08	0.12	0.44	0.66	1.60	2.65
Survival %								
<i>Week 13</i>	100	100	100	100	100	100	100	100
<i>Week 26</i>	99	100	98	100	100	100	100	100
<i>Week 51</i>	97	99	93	95	97	100	97	100
<i>Week 78</i>	91	89	85	73	90	92	83	90
<i>Week 104</i>	67	43	65	35	58	53	64	53
Body weight								
<i>Week 13</i>			(↓5%)		(↓1.2%)		(↓3.5%)	
<i>Week 26</i>			(↓2%)		(↓1.5%)		(↓3.9%)	
<i>Week 54</i>			(↓4%)		(↓4%)		(↓6%)	
<i>Week 78</i>			(↓10%)		(↓4.5%)		(↓8.9%)	
<i>Week 102</i>			(↓7%)		(↓2.6%)		(↓9%)	
Water consumption								
<i>Week 1-26</i>			(↓4%)	(↓7%)	(↓5.5%)	(↓5%)	(↓11%)	(↓13%)
<i>wk 31-105</i>			(↓12%)		(↓5%)	(↓6%)	(↓22%)	(↓13%)
Hematology:								
Leukocytes <i>Week 26/27</i>			-		-	-	(↓15%)	
<i>Wk 52</i>			-	-	-	-		(↓10%)
Monocytes <i>Wk 13</i>			-	-	-	-		(↓40%)
<i>Week 26/27</i>			-	-	-	-	(↑133%)	
<i>wk 52</i>				-	-	-	(↓29%)	
<i>wk 78</i>				-	-	-	(↓50%)	(↓64%)
<i>wk 104</i>			-	-	-	-	(↓50%)	
Eosinophils <i>Wk 13</i>			-	-	-	-		(↑61%)
<i>wk 26/27</i>			-	-	-	-	(↑100%)	(↑500%)
<i>wk 52</i>			-	-	-	-	(↑57%)	(↓36%)
<i>Wk 78</i>			-	-	-	-	(↓55%)	(↓62%)
Neutrophils <i>wk 78</i>			-	-	-	-		(↓20%)
<i>wk 104</i>			-	-	-	-	(↓20%)	(↑42%)
Urinalysis:								
Qualitative analysis:								
Volume			-	-	-	-	↓40% wk 78	↓41% wk 52
Blood occurrence			-	-	-	-	+ wk 78-103	+ wk 78
Wbcells			-	-	-	-	+ wk 78-103	+ wk 78
pH			-	-	-	-	5.0-8.0	
Organ weight: absolute								
<i>Interim kill</i>								
Liver			-	-	-	-		↓24%*
<i>Terminal kill</i>								
Adrenals			-	-	-	-	(↓47%)	(↓25%)
Pituitary			-	-	-	-	(↓35%)	(↓28%)
Organ weight relative:								
<i>Interim kill</i>								
Brain			-	-	-	-		↑19%*

Endpoints/dose	0		2 ppm		10 ppm		50 ppm	
	M	F	M	F	M	F	M	F
Calculated dose mg/kg bw/d			0.08	0.12	0.44	0.66	1.60	2.65
Gonads			-	-	-	-		↑55%*
Kidneys total			-	-	-	-		↑16%*
Terminal kill:								
Total adrenals			-	-	-	-	(↓38%)	(↓25%)
Pituitary			-	-	-	-	(↓29%)	(↓25%)
Necropsy findings								
N° rats terminal examined:	37	25	38	20	35	32	38	31
Skin subcutis mass (es) n°rats	7	16	6	18	11	24	9	25
Pituitary enlarged n°rats	1	5	3	2	3	8	4	10
All animals n° rats examined:	60	60	60	60	60	60	60	60
Skin subcutis mass (es) n°rats	14	33	14	40	19	43	15	41
Pituitary enlarged n°rats	2	7	3	4	5	8	5	10
Uterus distended enlarged	0	4	0	4	0	5	0	11

- : not measured; * statistically significantly different from control; () no statistical significance

Table B.6.5.1-2b: Incidence of histopathological findings in animals after MITC administration to rats.

Non neoplastic lesions/dose	N° affected rats							
	0		2 ppm		10 ppm		50 ppm	
	M	F	M	F	M	F	M	F
Calculated dose mg/kg bw/d	0	0	0.08	0.12	0.44	0.656	1.60	2.65
Rats dying during first 52 wk								
Lung congestion	1/3	1/1	1/4	2/4	1/2	0/0	3/3	0/0
All animals								
N° examined rats	60	60	55	59	55	60	60	60
Bone marrow necrosis					2			
Hyperplasia/granulopoiesis	2	0		5	2	3	3	8
Harderian gland adenitis	13	21	4	16	4	11	14	24
Kidney microcalculi	0	14	1	15	1	5	1	21
Renal scarring		2		1			7	
Pyelitis	4	2	2	3	2	1	7	3
Liver:								
Fatty vacuolation	9	17	9	21	2	15	14	23
Leucocyte infiltration	6	4	4	10	2	4	11	12
Biliary proliferation	11	5	3	5	4	6	13	10
Necrosis/scarring	1	2	2	3	3	1	2	4
Pulmonary artery calcification	14	8	6	12	8	5	16	15
Mammary gland								
Lobular/acinar hyperplasia	7	11	2	8	5	2	5	9
Ovary cysts		6		6		9		13
Spleen hyperplasia/↑haematopoiesis	8	3	5	6	2	5	5	11
Thyroid C cell hyperplasia	3	12	2	8	1	7	3	5

Neoplastic findings (Table B.6.5.1-3b):

Neoplastic lesions were more prevalent in the rats dying during the first year of study. However, the distribution and frequency of individual neoplasm failed to establish a drug relationship. The group with the most numerous tumors was the low dose group. At interim sacrifice, three neoplasms were observed. No evidence of compound-induced oncogenicity was currently observed at interim sacrifice.

In the terminal kill, the high dose group had slightly more females with multiple benign mammary tumors than the control group: this difference did not achieve statistical significance, and was not reflected in any other category of mammary tumor in either terminal, incidental or pooled data.

Overall, tumors of the endocrine glands accounted for a high proportion of the tumors found in this study. More than half of the female rats, and many male rats had pituitary tumors. Thyroid C-cells tumors were found in approximately 10% of rats of either sex, and pheochromocytoma occurred in more than 10% of males but fewer females. Mammary gland tumors were also common occurring in more than half of the female rats. The difference did not achieve statistical significance.

Table B.6.5.1-3b: Incidence of neoplastic lesions occurring as a function of time after MITC administration to rats.

Neoplastic lesions/dose	N° affected rats/n° examined							
	0		2 ppm		10 ppm		50 ppm	
	M	F	M	F	M	F	M	F
Calculated dose mg/kg bw/d			0.08	0.12	0.44	0.66	1.60	2.65
Rats dying before study termination								
Mammary gland								
Benign single tumor	0/23	11/35	0/22	8/40	1/25	8/28	0/22	8/29
Benign multiple tumor	0/23	5/35	0/22	7/40	0/25	6/28	0/22	3/29
malignant single tumor		1/35		2/40		1/28		
Malignant tumor single/benign				1/24		3/28		2/29
Malignant tumor multiple /benign				1/24				
Pituitary adenoma	8/23	27/35	7/22	31/40	6/25	24/28	5/22	21/29
carcinoma								1/29
All animals								
Pituitary								
adenoma	19/60	44/60	15/55	39/59	12/55	41/60	20/60	38/60
adenocarcinoma				1/59				2/60
Thyroid C cell adenoma	6/60	6/60	2/55	3/59	2/55	3/60	7/60	8/60
Thyroid C cell carcinoma	3/60	2/60	1/55			2/60	5/60	2/60
Follicular adenoma/carcinoma		1/60			1/55	1/60		
Mammary gland								
Benign single tumor	2/60	20/60	0	15/59	1/55	17/60	0	16/60
Benign multiple tumor	0	11/60	0	15/59	0	18/60	0	18/60
malignant single tumor	0	1/60	0	3/59	0	1/60	0	
Malignant tumor single/benign	0		0	1/59	0	5/60	0	2/60
Malignant tumor multiple /benign				1/59				
Adrenals								
pheochromocytoma	9/60	4/60	2/55	1/59	2/55	0/60	7/60	1/60
Cortical adenoma						1/60		
Subcutaneous tissue								
Fibroma- single	3/60	2/60	3/55		2/55	6/60	8/60	3/60

Conclusion:

A NOAEL=10 ppm (0.44 mg/kg bw/d) is proposed. This proposal takes into account the reduced body weight of male rats, which is more important at top dose at the end of the study period. Parameters such as alteration of white blood cells, some histopathological findings (bone marrow hyperplasia, increased kidney microcalculi, liver effects, and spleen hyperplasia/ increased haematopoiesis etc...) seen at 50 ppm (1.6 mg/kg bw/d) which could be related to MITC exposure.

MITC is not carcinogenic under these experimental conditions.

Already Peer Reviewed, EFSA 2008

During Peer Review, the use of proposed MITC intake in the 2 year rat study with MITC was discussed.

The acceptability of the study was questioned by EFSA, because of the instability of MITC in solution.

The ingested doses were calculated using a correction factor of 87.7%. This figure was obtained using the analytical measurements of the 10 ppm dose group, in the drinking water bottles used during the greatest part of the study. Thus, the NOAEL was converted to 0.44 mkd. In the M&M section of the DAR, it was explained that the concentration of MITC in the different bottles used was regularly determined at all dose levels, and fresh solutions were prepared daily until a new supply of MITC was available. This procedure helped to avoid a too fast degradation.

It could be argued whether the use of 82%, which is the overall mean value, taking into account the mean recovery over 3 days after preparation, would be more suitable. In this case, the proposed NOAEL would be revised downwards to 0.37 mkd i.o. 0.44 mkd. In both cases, a reference dose would then be rounded to 0.004 mkd. In addition, the actual proposition for the ADI is based upon the dog (90d drinking water) study, which was the most sensitive species. Thus, a recalculation of the NOAEL (and a fortiori of the lowest dose) would not be critical in the risk evaluation.

At the highest dose of the carcinogenicity study, mild toxicity was observed but no treatment-related neoplasms were reported. Therefore, the long-term rat study with MITC was considered scientifically acceptable.

GLP status: yes (no attest of competent authority)

Experimental protocol: not fully in agreement with Directive 87/302/CEE Annex V.3 or OECD 453 (1981).

Deviation from official protocol:

Number of animal is limited to 60 (2 year) rats/sex/dose + five (1 year) and five (1 year + reversibility ad 4 weeks) for control and highest dose. Three dose levels were used, identical for chronic toxicity and carcinogenicity, without adverse effects. Ornithine decarboxylase was not measured. Absence of raw data for clinical signs.

Material and methods: 60 CD rats/sex/dose received MITC (batch n° 28 466: 95.58%; batch n° 29 482: 95.41%; batch n° 29 482: 95.36% and batch n° 29 482: 96.06%) in their drinking water for 104 weeks at 2, 10 or 50 ppm. The different batches tested in this study contain CS₂ (0.85-1.33%), dimethyl sulfide (0.11-1.51%), dimethyldisulfide (2.85-3.03%) and methylthiocyanate (traces-0.12percentage).

Additional groups of 10 animals were used as control and treated with top dose from which five were sacrificed after 53 weeks. A further 5 rats of each sex from these groups were then given untreated water for 4 weeks before being killed and necropsied in order to assess reversibility.

Over the whole study, the mean concentrations of MITC formulations given to the animals (taken from drinking bottle analysis) were 1.3 ppm, 8.2 ppm and 41.6 ppm at nominal dose levels of 2 ppm, 10 ppm and 50 ppm, respectively, corresponding to 65%, 82% and 83% of the nominal concentration. The majority of the study was conducted with doses of 0.08, 0.37 and 1.6 mg/kg bw/d in males and with doses of 0.12, 0.56 and 2.65 mg/kg bw/d in females respectively. The mean analytical concentrations at 10 ppm were 87.7% of nominal in polyethylene bottles, which were used over the majority of the study period. In addition, the report states that fresh solutions were prepared daily until a new supply of MITC was available. This procedure helped to avoid a too fast degradation and additionally confirms that the use of a correction factor of 87.7% is appropriate at a dose level of 10 ppm. The dose levels presented in mg/kg bw/d were calculated based on the mean body weights and mean water consumption rates determined over the study period in male and female animals. These dose levels were corrected for the mean analytical concentrations found resulting in actual doses of 0.44 and 0.66 mg/kg bw/d in male and female rats respectively. A very low level of contamination with an impurity was observed in the control formulation for weeks 23 and 36 and was attributed to an impurity in one of the solvents used.

The study is accepted.

B.6.5.2b Carcinogenicity study in the rat (Annex IIA 5.5)

See point B.6.5.1b.

B.6.5.3b Carcinogenicity study in the mouse (Annex IIA 5.5)

- **Methyl isothiocyanate (MITC): 2 year oral toxicity and oncogenicity study with methyl isothiocyanate in albino mice (106 week final report) (Sato et al, 1980)**
- **Addendum to final report (Kashima and Sato, 1985) and Methyl isothiocyanate: two-year oral toxicity study in albino mice T52 amend 1, T52 addendum (Hiraide, 1988)**

Findings

Mortality: was increased in the last part of the experiment. However, there were no differences in the number of deaths between the experimental groups and the control group.

Clinical signs: at 80 and 200 ppm, mice had slightly raised hair and a dull coat about 30 days after beginning of exposure.

Skin fibrosarcoma were seen in prematurely died animals at top dose. Statistical significance was not attained when sexes were considered separately but significance was reached at top dose when the data were combined. However, as the incidence did not increase with duration of exposure, MITC should not be considered as carcinogenic in mice.

[illegible]

Endpoint/Dose	0		5		20		80		200 ppm	
	M	F	M	F	M	F	M	F	M	F
MITC intake mg/kg bw/d	0	0	0.82	0.91	3.3	3.66	11.83	13.03	25.71	29.03
lymphocyte							↓33%*		↓37%*	
Segmented leucocytes							↑64%*		↑74%*	
week 106										
Basophilic cells							↑266%*		↑166%*	
Clinical chemistry										
Week 26										
Total protein							↓7%*	↓9%*	↓7%*	↓10%*
BUN							↓20%*		↓20%*	
cholesterol										↓20%*
Week 106										
GOT										↑25%*
Urinalysis										
Week 52										
K ⁺							↑62%*	↑53%*		↑49%*
Organ weight relative:										
Week 26										
Pancreas										↑25%*
Heart									↑11%*	
Kidney									↑14%*	↑37%*
Adrenal gland										↑40%*
Brain										
Thymus										↑81%*
Pituitary gland										↑38%*
Spleen										↑34%*
Week 52										
Thyroid gland							↑82%*	↑57%*	↑67%*	↑46%*
Spleen							↑80%*		↑57%*	
pituitary								↑95%*	↑52%*	↑57%*
Week 106										
Bladder							↑10%*		↑33%*	
Adrenal gland								↑26%*		↑42%*
Pituitary										↑27%*
thyroid										↑33%*
Autopsy findings wk 106										
Urinary bladder distended	0%		15%		10%		6.6%		24%	
Histopathology in mice dying during the study: n° affected mice										
N° mice	35	37	25	38	29	37	28	39	37	37
Spleen small round cell infiltration	1	0	1	0	1	0	1	0	5	0
Cellular infiltration	0	0	0	1	0	2	0	2	0	12
Small intestine amyloid degeneration	9	0	9	0	13	0	7	0	15	0
Skin fibrosarcoma	0	0	0	0	0	1	0	0	2	3
Histopathology at wk 106: % affected mice										
n° mice	23	21	33	20	29	21	30	19	21	21

Endpoint/Dose	0		5		20		80		200 ppm	
MITC intake mg/kg bw/d	M	F	M	F	M	F	M	F	M	F
	0	0	0.82	0.91	3.3	3.66	11.83	13.03	25.71	29.03
Pulmonary adenocarcinoma	0	14%	0	15%	0	19%	0	16%	0	33%
Liver small round cell infiltration	0	23%	0	25%	0	33%	0	31%	0	42%
Kidney small round cell infiltr.	0	28%	0	30%	0	24%	0	16%	0	62%
Amyloid degen.	19%	24%	30	15	48	24	36	42	57%	5%
bladder small round cell infiltration	0	19%	0	20%	0	38%	0	21%	0	42%
Bone marrow small round cell infiltration	0	0%	0	5%	0	4.5%	0	5%	0	24%
Ovary cysts	-	9.5%	-	20%	-	14%	-	26%	-	47%
Overall tumor incidence: n° affected mice/n° examined										
Liver carcinoma	2/64	1/64	4/64	0/64	1/64	0/64	4/64	1/63	2/63	2/64
Skin squamous cell carcinoma	0/64	-	0/64	-	0/64	-	0/64	-	1/63	-
Skin fibrosarcoma	0/64	0/64	0/64	0/64	0/64	0/64	0/64	0/63	2/63 (3.17%)	3/64 (4.69%)
Mesenteric lymph node Leukemia	6/64	27/64	8/64	29/64	9/64	24/64	13/64	24/63	5/63	30/64
Lung adenocarcinoma	19/64	8/64	19/64	5/64	19/64	11/64	22/64	7/63	18/63	10/64

* Statistically significantly different from control

Conclusion: a NOAEL can be set at 20 ppm (3.3 mg/kg bw/d) taking into account the different slight effects seen at 80 ppm (12 mg/kg bw/d) such as increased incidence of clinical signs, slight decreased body weight and body weight gain, slight effects in blood and altered organ weights. MITC should not be considered as carcinogenic in mice.

Already Peer Reviewed, EFSA 2008

(a) Point of clarification during Peer Review: Applicant to provide historical control data for skin fibrosarcoma in albino mice

In the study, it is reported that these mice died during the study. The timepoint of death of 2 ♂ (at d748 and d639) and 3 ♀ (at d681, d664 and d628) were confirmed by the notifier, suggesting the occurrence of the tumours only at the end of the study.

Historical control data on a very limited number of studies (n=3), were submitted for these tumours (see table with fibrosarcoma incidence in the skin/subcutal tissue of control ICR-JCL mice). Maximal incidences were 2.9% (♂)-4.4% (♀), which were comparable with the incidences in the study (3.2% in ♂ and 4.7% in ♀). As the tumours (which were graded benign) occurred only in old mice, that the incidences were only marginally higher than the maximal incidence in a time-matched study, RMS considered the findings insufficient to consider the compound carcinogenic.

	♂				♀			
study	a	b	c	total	a	b	c	total
week	87	68	104		98	104	104	
survival	34	67	50	151	26	23	54	103

occurrence	1	1	0	2	0	1	0	1
incidence (%)	2.94	1.49	0	1.32	0	4.35	0	0.97

Study a: 98-wk chronic toxicity study (1981);

Study b: 24-month chronic toxicity study (1983);

Study c: 24-month chronic toxicity study (1971);

Source: Nippon Experimental Medical Research Institute Co, Ltd.

(b) The relevant NOAEL of the 2 year drinking study in mice with metam sodium (Horner, 1994) was discussed during Peer Review

The incidence of angiosarcoma at the mid dose (0.074 mg/L = 7.2 mkd) was considered relevant, to set the carcinogenicity NOAEL at the lowest dose = 0.019 mg/L (1.9 mkd). The incidence in males at low dose is slightly outside the HCD, but the incidence in control animals is higher than the HCD. The systemic NOAEL was established at the same level, taking into account the rise in urinary bladder epithelial hyperplasia and hepatic necrosis.

After reconsidering the data, the notifier agreed with RMS.

GLP status: yes (no attest of competent authority)

Guidelines: not fully in agreement with Directive 87/302/CEE Annex V B or OECD test guideline 451 (1981).

Deviation from official protocol: Test is performed on 48 animals/sex/dose at 2 year and six at 6 and 12 months. No blood smears but hematology, biochemical tests and urinalysis. Histopathology of trachea and aorta, esophagus, salivary glands, pancreas, mammary glands and bone marrow not performed. Raw data of clinical signs not provided.

Material and methods: MITC (lot n° MS 25206; 93.14%) was administered ad libitum in drinking water to 70 mice ICR: JCL (Japan) /sex/dose at 5, 20, 80 and 200 ppm. Control animals received water alone. Six mice/sex/dose were killed 26 weeks and 52 weeks after the beginning of exposure. Remaining animals were sacrificed after 106 weeks. Fresh solution was prepared every day.

Statistics: all the data were analyzed by means of t test and the incidence of tumor was determined by mean of X² test.

The study is accepted.

B.6.5.4 Mechanism of action and supporting data (Annex IIA 5.5)

- Assessment of the carcinogenic potential of metam sodium (Pistel, 1994)

Bladder changes in mice after exposure to metam sodium:

Bladder carcinogenesis in mice is an uncommon spontaneous process. In the mice carcinogenicity study, the increased incidence is not statistically significant but the reason why this tumor nevertheless may be associated with metam exposure is that in both the 3-month study as well as in the carcinogenicity study, histopathological changes in the bladder were clearly related to the administration of metam sodium.

- In the 3-month study, the first changes in the bladder were eosinophilic granules at 0.088 mg/ml. At the two higher doses of 0.35 and 0.62 mg/ml, cystitis and mucosal hyperplasia were observed in both males and females. Although the toxicological significance of the eosinophilic granules is uncertain, cystitis and mucosal hyperplasia clearly indicate that metam sodium or one of its metabolites is damaging the urinary bladder epithelium. Epithelial hyperplasia was observed at 0.074 and 0.23 mg/ml in the carcinogenicity study in mice. In addition, changes such as submucosal inflammatory infiltration and mononuclear cell infiltration were associated with top dose. From these data, it can be derived that the bladder damage noted in the 3-month study is occurring at lower dose levels in the long-term study, indicating a progression of the damage over time. Increased cell proliferation in the bladder is occurring because of the metam sodium administration.
- Consequently, it can thus be derived that a hyperplastic process in the bladder is very likely to have occurred during the major part of the long-term mouse study. Chronic hyperplasia is frequently associated with the occurrence of carcinogenic changes in the affected organ. Therefore, it is not surprising that in the long-term study an increase in the incidence of neoplastic changes was observed. The extremely low incidence, however, does indicate that the transition from hyperplasia to neoplasia occurs very rarely.

Bladder changes could result of a toxic action of one of the urinary metabolites of metam sodium. When comparing the results of the kinetic studies with metam sodium and MITC, it is obvious that the MITC metabolites are excreted mainly via the urine. It is interesting to note that MITC, when tested at comparably high dose levels (approximately 30 to 36 mg/kg bw/d) did not induce clear changes in the urinary bladder of mice. Moreover, MITC did not induce bladder carcinogenesis. It may thus be concluded that a urinary metabolite of metam sodium, not related to MITC or its metabolites, is responsible for the bladder damages.

Increased incidence of angiosarcoma:

The administration of 0.62 mg/ml metam sodium over 24 months to mice was clearly associated with an increased incidence of angiosarcoma. The major part of these tumors was found in the spleen, with some evidence for increased incidences in the liver, bone marrow and subcutaneous tissues. The common denominator of the above-mentioned tissues and organs is that the blood vessels branch into many capillary resulting in both an increase in epithelial blood vessel surface, a rich supply of blood as well as a reduction of the velocity of blood flow.

Non-neoplastic changes in the vascular system were not noted in the mouse studies with metam sodium. However, the administration of metam sodium did affect hematological parameters, especially in the 3-month mouse study. These changes (reduced Hb, Ht and RBCcount) indicate a hemolytic effect of the test substance and occurred at dose levels of 0.35mg/ml and higher in males and of 0.088 mg/ml and higher in females. In the 24-month study, hematological parameters were much less affected. Nevertheless, signs of hemolytic effect of the test substance were also found in this study (slightly reduced RBC count and slight hemosiderosis of the spleen). The above-mentioned changes of hematological parameters suggest the possibility that the effects of metam sodium on the blood may be associated with the increased incidence of tumors of the blood vessels.

The results of the kinetic studies with metam sodium in the rat demonstrated a profound difference in the rate of formation of MITC at the high and low dose level. From the data it may be concluded that at high dose levels the metabolic capacity is overwhelmed, resulting in an increasing amount of unmetabolized MITC at higher dose levels. If a similar situation would be found for the kinetics of metam sodium in the mouse, then it may be argued that the results of high dose levels cannot be extrapolated linearly to low dose levels because of significant differences in the metabolic pattern.

The author did not receive studies performed with MITC but concluded that, derived from published studies, it should be noted that one of the main metabolites of metam sodium, MITC, was not found to be carcinogenic in mice at comparably high dose levels.

B.6.5.5 Summary of long-term toxicity and carcinogenicity (Annex IIA 5.5)

Metam sodium was administered to rats in drinking water for 2 years. At 0.19 mg/ml, rats showed signs of systemic toxicity indicated by reduced bodyweight gain, specific lesion within the nasal passages, and changes in some hematology and spleen (hemosiderin depots) parameters and a slight increase in the severity but not of incidence of degenerative myopathy of the voluntary muscle. There were a few changes associated with the 0.056 mg/ml dose level, which were consistent with, but less marked than effects at the high dose. The incidence of hemangiosarcoma in males in the mesenteric lymph nodes was slightly higher than the control at the intermediate dose of 0.056 mg/ml but this effect was not observed at top dose. In view of the overall results obtained in this carcinogenicity study, it can be concluded that metam sodium is not carcinogenic in rats. A NOAEL was established at 0.019 mg/ml.

Mice received in their drinking water metam sodium at 0.019, 0.074 or 0.23 mg/ml for 24 months.

Both male and female mice had urinary bladder lesions and pathological significant changes were mainly present at 0.23 mg/ml. At 0.074 and 0.23 mg/ml, there was an increased incidence of angiosarcoma in males and females. Metam sodium is carcinogenic in mice and the symbol **Xn** and risk phrase **R40** is proposed (limited evidence of a carcinogenic effect). A NOAEL systemic toxicity and carcinogenicity = 0.019 mg/ml is proposed taking into account the increased incidence of urinary bladder epithelial hyperplasia, hepatocytic necrosis and increased incidence of angiosarcoma at 0.074 mg/ml.

The increased incidence of bladder neoplasia reported at top dose is associated with the treatment of metam sodium. The reason for this increase could be related to the induction of chronic hyperplasia in this organ. The extremely low incidence of neoplasia, however, indicates that the transition from hyperplasia to neoplasia is a rather rare event.

Table B.6.5.5-1: Summary of long-term toxicity and carcinogenicity of Metam

Type of test Test species	Test substance purity	Results			References
		NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Symptoms	
Rat, 104 week drinking water study at 0.019, 0.056 and 0.19 mg/ml	Batch n° BAS/005/OON 90-2; 525.54 g/L = 43.148% w/w	0.019 mg/ml = 1.5 mg/kg bw/d	0.056 mg/ml = 4.3 mg/kg bw/d	nasal passages toxicity, ↓ body weight, changes in some hematological parameters	Allen, 1991 Rattray, 1994
Mice study at 0.019, 0.074 or 0.23 mg/ml in drinking water for 24 months	Batch n° BAS/005/OON 90-2; 525.54 g/L (43.148%)	0.019 mg/ml (1.9 mg/kg bw/d)	0.074 mg/ml (7.2 mg/kg bw/d)	Urinary bladder hyperplasia, ↑ incidence of angiosarcoma, and at top dose: urinary bladder neoplasm Xn, R40	Horner, 1994 Pistel, 1994

Rats received MITC via drinking water at 2, 10 or 50 ppm over 104 weeks. Reduced body weight of male rats, was more important at top dose at the end of the study period, white blood cell parameters, histopathological findings such as bone marrow hyperplasia, increased kidney microcalculi, liver effects, and spleen hyperplasia/increased haematopoiesis were reported at 50 ppm (1.6 mg/kg bw/d) and could be related to MITC exposure. MITC is not carcinogenic under these experimental conditions. A NOAEL=10 ppm (0.44 mg/kg bw/d) is proposed.

Mice received MITC in drinking water at 5, 20, 80 and 200 ppm. A NOAEL can be set at 20 ppm (3.3 mg/kg bw/d) taking into account the different slight effects seen at 80 ppm (12 mg/kg bw/d) such as the increased incidence of clinical signs, slight decreased body weight and body weight gain, slight effects in blood and altered organ weights. MITC is not carcinogenic in mice.

Table B.6.5.5-2: Summary of long-term toxicity and carcinogenicity of MITC

Type of test Test species	Test substance purity	Results			References
		NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Symptoms	
104 weeks rat study at 2, 10 or 50 ppm in drinking water	batch n° 28 166: 95.58%; batch n° 29 482: 95.41%; batch n° 29 482: 95.36% and batch n° 29 482: 96.06%	10 ppm (0.44 mg/kg bw/d)	50 ppm (1.6 mg/kg bw/d)	↓ body weight, altered WBC parameters, bone marrow hyperplasia, ↑ kidney microcalculi, liver effects, and spleen hyperplasia/ increased haematopoiesis	Brown, 1984 Brown, 1988 Iwan, Kaiser, and Hollihn, 1988
Mice, drinking water study at 5, 20, 80 and 200ppm	lot n° MS 25206; 93.14%	20 ppm (3.3 mg/kg bw/d)	80 ppm (12 mg/kg bw/d)	↑ clinical signs, ↓ body weight, body weight gain, slight effects in blood and altered organ weights	Sato et al, 1980 Shigeshi Toyoshima, 1982 Kashima and Sato, 1985 Hiraide, 1988

B.6.6a Reproductive toxicity of Metam sodium (Annex IIA 5.6)

B.6.6.1a Two generation reproductive toxicity in the rat (Annex IIA 5.6.1)

- Metam sodium: multigeneration study in the rat (Milburn, 1993)

Findings: see Table B.6.6.1.1-a:

Although the drinking water preparations were stable for 24 hr in most cases, there were a small number of preparations where the metam sodium concentration was significantly lower after 24 hr. The overall mean achieved concentrations were 102, 96.7 and 100% of the nominal at the 0.01, 0.03 and 0.1 mg/ml concentrations respectively.

Mortality: was not attributable to metam sodium.

Clinical signs: there was no evidence of an effect of metam sodium in either generation.

Body weight:

F0 parents:

The weight of males at 0.1 mg/ml showed decreases on occasion, although differences were marginal. Females at 0.1 mg/ml had statistically significantly lower bw than controls although differences were marginal.

Pregnancy and lactation weight was reduced in females at 0.1 mg/ml. A marginal effect was seen in females at 0.03 mg/ml.

F1 parents:

Pre-mating bw was reduced in males and females receiving 0.1 mg/ml. Pregnancy and lactation weights were reduced in females receiving 0.1 mg/ml.

Food consumption was marginally reduced at top dose in males and females F0 and F1 parents. There were occasional reductions in F1 females at 0.01 and 0.03 mg/ml but there was no dose relationship.

Water consumption:

F0 parents: was reduced in males and females at 0.1 and 0.03 mg/ml during the pre-mating period. During pregnancy, a reduction was reported in the 0.1 and 0.03 mg/ml group but not in the 0.01 mg/ml.

During lactation, a reduction was seen at 0.1 mg/ml and to a lesser extent in the 0.03 mg/ml group.

F1 parents: was reduced during the pre-mating period in males and females receiving 0.1 mg/ml. There was a consistent reduction in females receiving 0.03 mg/ml. There was a marginal decrease in females receiving 0.01 mg/ml during the early part of the pre-mating period. Water consumption was reduced during pregnancy in a dose-related manner in all dose groups.

During lactation, a reduction was seen in all animals at 0.1 mg/ml. In the 0.01 mg/ml group, there was a reduction at all time points and at 0.03 mg/ml, during week 1 only. The differences from controls were not dose-related.

Reproductive performance: metam sodium did not affect the pre-coital and gestation period.

Male and female fertility was good with 29-30 litters of a possible 30 produced in each group.

The percentage of pups live born was in excess of 94% for each group in both generations. The number of whole litter losses was low, and there were no litter losses in the high dose group.

Females of the top dose group, which did not have nasal lesion, were those who failed to produce litter or had small litters (1 with 6 live offspring's (F0) and 2 with 2 live offspring by day 5 post partum (F1)).

Males receiving 0.1 mg/ml or females at lower doses did not show nasal cavity abnormalities.

The number of F0 rats suspected to be infertile was small i.e. five males only, and was unrelated to treatment: the few changes observed in the reproductive organs of these rats were example of common spontaneous lesions. The number of F1 males suspected to be infertile was slightly increased in all treated groups (4/group) compared to control (2/group). However, no lesions were detected to account for infertility. One female of control and one of the top doses failed to produce litters. No microscopic changes were detected in the abnormal pituitaries and in the twisted snout from one F1 male.

Organ weight: there was no effect on the weight of testes or epididymides of F0 or F1 parents at any dose level.

Macroscopic findings:

F0 adults: red spots were observed on the pituitary gland of two females at 0.1 mg/ml.

F1 adults: one male at top dose had a twisted snout.

Microscopic findings:

Nasal cavity: see Table B.6.6.1.1-2a.

F0 and F1 adults:

Minimal to marked Bowman's gland duct hypertrophy with loss of alveolar cells was detected in the olfactory mucosa lining the nasal septum and turbinate bones at all levels of the nasal cavity in 29/30 F0 and 27/30 F1 females at top dose.

The F0 rat in which this was not present had ducts of Bowman's gland greatly increased in prominence in both the epithelium and *lamina propria*. Duct hypertrophy was accompanied by loss of alveolar cells in the lamina propria. The lesion was mainly confined to the nasal septum and dorsal arch with occasional foci on the opposing turbinate. 14 of the F0 females also showed minimal or slight dilatation of the ducts of Bowman's glands.

The change in the Bowman's gland was accompanied in all affected rats by minimal to marked degeneration/disorganization/atrophy of the olfactory epithelium which comprised vacuolation and separation of cells in the basal layers of the olfactory epithelium with a few necrotic cells evident, loss of normal cellular arrangement and apical cytoplasm and reduction in n° of cell layers. These changes occurred together in variable combinations in all affected rats. The incidence and severity of degeneration/disorganization/atrophy was considerably increased in the top dose group compared with control i.e. 29/30 affected F0 and 27/30 F1 with minimal to marked severity compared with only 4 minimally affected females in the control group. One female receiving 0.1 mg/ml showed minimal hyperplasia of the olfactory epithelium in addition to the other changes described; the hyperplasia was minimal or slight and confined to a small area of the dorsal arch in the posterior region of the nasal cavity.

Offspring: individual pup weight, total litter weight and pup weight gain was reduced in the 0.1 mg group in both generations. Although F2A total litter weights were lower than those observed in controls for the 0.01 mg/ml, there was no similar finding for the 0.03 mg/ml. In addition, when adjusting total litter weight for F2A litters for litter size the difference was no longer evident in the 0.01 mg/ml group indicating that the difference from control is not treatment related. There was no effect on the body weight of pups at 0.01 or 0.03 mg/ml. There was no indication of a change in the nasal passage of offspring of either generation at any dose level.

Survival was good and there was no evidence of an adverse effect of metam sodium at any dose level. Litter sizes were similar in all groups for the F1A litter.

The number of pups/litter in the 0.01 mg/ml for the F2A litter was slightly lower than control but in the absence of a similar finding in the higher dose groups, this is probably incidental.

Clinical observations and findings at evisceration: there was no effect on the type or incidence of clinical observations in F1A or F2A offspring. There was no evidence of an effect on the findings in pups found dead up to and including 18 days of age.

Organ weight: there was no effect on the weight of testes or epididymides of F1A or F2A offspring at any dose level.

Microscopic findings:

F1A pups: nasal cavities did not show abnormalities, which could be related to treatment. Minor degenerative and inflammatory changes were detected in the respiratory mucosa of the majority of pups but the incidence was similar in all groups. No ocular tissues were detected in the female pup w/o eye: bilateral anophthalmia seems to occur spontaneously in the Alpk: APfSD rat and this isolated incidence in one generation only are considered to be of no toxicological significance.

F2A pups: nasal cavities from control and high dose pups were comparable.

Table B.6.6.1.1-1a: 2-generation rat study with metam sodium in drinking water.

	0		0.01		0.03		0.1 mg/ml	
	M	F	M	F	M	F	M	F
mg/kg bw/d F0 and F1	0	0	1.4-1.7	2.3-2.86	4-4.6	6.7-7.5	12.3-14	19.6-21.6
Body weight F0:								
prematuring parent							↓2% * wk 3-9	↓3% * wk 2-11
during gestation								
Day 1,8,22							↓3% *, ↓1% *, ↓8% *	
during lactation								
Day 1,11,16,22,29							↓6% *, ↓3% *, ↓3% *, ↓6% *, ↓3% *	

	0		0.01		0.03		0.1 mg/ml	
	M	F	M	F	M	F	M	F
Body weight F1:								
Premating parent							↓4-6% * wk 2-11	↓3-6% * wk 2-11
during gestation								
Day 1,8,15,22							↓5% *, ↓1% *, ↓2% *, ↓6% *	
during lactation								
Day 1,5,11,16,22,29							↓11% *, ↓10% *, ↓2% *, ↓4% *, ↓5% *, ↓4% *	
Food consumption								
F0 parent premating							↓3-4% * wk 1-5	↓3-4% * wk 1,2,5,6,7,9,10
F1 parent premating				↓4-5%* wk 1,2,4		↓4-5%* wk 1,2,4	↓4-7% * wk 1,2,5,6	↓4-7% * wk 1-9
Water consumption F0								
Parent premating					↓5-14%* wk 1-6	↓11-26%* wk 1-5; ↓23-16% wk 5-8	↓8-29%* wk 1-5; ↓24-16%* wk 6-10	↓5-40%* wk 1-10
during gestation					↓14%* wk 2-3		↓34-36%* wk 1-3	
during lactation					↓10,17,13%* wk 2,3		↓20%* wk 1-4	
Water consumption F1								
parent Premating				↓6-12%* wk 1-4		↓10-31%* wk 1-10	↓9-19%* wk 1-10	↓15-46%* wk 1-10
during gestation				↓15-11%* wk 2-3		↓17,23,14%* wk 1-3	↓44-36%* wk 1-3	
during lactation				↓12-10%* wk 1-4		↓9%* wk 1	↓23-10%* wk 1-4	
Fertility:								
F1A litter	29/30	30/30	28/30	30/30	28/30	30/30	30/30	30/30
F2A litter	28/29	29/29	26/30	30/30	26/30	30/30	26/29	29/30
N° pups live born:								
F1A litter	341		359		316		348	
F2A litter	353		324		361		332	
N° litters with all pups born live:								
F1A	26		24		24		26	
F2A	20		25		26		22	
Whole litter losses								
F1A	1/30		2/30		1/30		0/30	
F2A	2/29		0/30		0/30		0/29	
Pup/litter survival								
F1A d1-22	317/16		309/16		296/17		323/17	
F2A d1-22	293/13		284/16		331/19		311/22	
Litter size Day1/day 29								
F1A	11.7/10.9		12/11		10.9/10.2		11.6/10.7	
F2A	12/10.9		10.8/9.4		12/11		11.4/10.7	
Pup body weight:								
F1A							↓6-5%*	↓5%*

	0		0.01		0.03		0.1 mg/ml	
	M	F	M	F	M	F	M	F
							d 22-29	d 29
F2A							↓8-9% d11-29	↓5-8%* d5-29
Litter body weight								
F1A			No compound related effect					
F2A			↓11%* d1 ↓14%* d11 ↓12%* d16 ↓13%* d22				↓11%* d16 ↓14%* d22	
Adjusted litter body weight:								
F2A							↓8-11%* D5-29	
Pup weight gain:								
F1a Day 1-29							↓5%	↓6%
F2a Day 1-29							↓10%	↓10%

* Statistically significantly different from control

Table B.6.6.1.1-2a: 2-generation rat study with metam sodium in drinking water: microscopic findings.

Microscopic findings/dose	0		0.01		0.03		0.1 mg/ml	
	M	F	M	F	M	F	M	F
Nasal cavity:								
F0 parent								
n° examined	30	30	0	0	0	30	30	30
Bowman's gland duct hypertrophy + loss alveolar cells: minimal/slight/moderate/marked n° affected	0	0				0	0	2/6/15/6
Dilatation total	0	0				0	0	14
Olfactory epithelium disorganization/degeneration/atrophy: minimal/slight/moderate/marked n° affected	0	4/0/0/0				2/0/0/0		3/10/13/3
Hyperplasia	0	0				0	0	1
F1 parent								
n° examined	30	29	3	0	4	30	30	29
Bowman's gland duct hypertrophy + loss alveolar cells: minimal/slight/moderate/marked n° affected	0	0	0		0	0	0	2/10/12/3
Dilatation total	0	0	0		0	0	0	10
Olfactory epithelium disorganization/degeneration/atrophy: minimal/slight/moderate/marked n° affected	0	3/0/0/0				1/0/0/0		5/14/5/2
Hyperplasia total	0	0				0	0	3

Conclusion:

Metam sodium affected nasal passage of the majority of F0 and F1 adult females and more specifically Bowman's gland which were hypertrophied at 0.1 mg/ml (= 17 mg/kg bw/d). There was no effect on the nasal passages of adult males or offspring. This suggests that the nasal lesion was a direct consequence of the increased dose received by females during lactation. Water consumption was reduced at top dose and was accompanied by slight reductions in body weight, which were most evident during gestation, and lactation in adults.

Effects in pups were a slight reduction in individual pup weight and in total litter weight in the 0.1 mg group in both generations. There was no indication of a change in the nasal passage of offspring of either generation at any dose level.

NOAEL reproduction toxicity > 0.1 mg/ml = 12 mg/kg bw/d.

NOAEL fetotoxicity = 0.03 mg/ml = 4 mg/kg bw/d is proposed based on the decreased pup and litter weight observed at 0.1 mg/ml (12.3 mg/kg bw/d).

NOAEL parental toxicity = 0.03 mg/ml (= 4 mg/kg bw/d) is proposed based on the decreased body weight during prenatally, gestation and lactation reported at 0.1 mg/ml.

GLP status: yes (no attest of competent authority)

Experimental protocol not fully in agreement with Directive 87/302/CEE Annex V B or OECD test guideline 416 (2001-1983).

Deviation from official protocol: initially, histological examination was to be restricted to nasal cavities from adults and pups in control and top dose and reproductive organs in all groups that were infertile. However, nasal cavities of intermediate animals were also examined. Pituitary glands limited to two females. All remaining tissues were stored.

Material and methods: 30 Alp: APfSD rats/sex (FO parents) were given drinking water containing 0.01, 0.03 or 0.1 mg/ml metam sodium (batch ref BAS/005/00N; 526 g/L or 43.148%/w; CTL n0 Y06930/008). After 10 weeks, rats were mated and allowed to rear the ensuing F1A litters to weaning. The breeding programme was repeated with the F1 parents selected from the F1A offspring to produce F2A litters after a 10-week pre-mating period.

Water was adjusted to approximately pH 8-9. A fresh bottle was supplied daily. Drinking water was analyzed two x/week in the first 2 weeks of the study and then at least one batch/month.

Statistics: analysis of covariance and variance were used. Fisher exact test, double arsine transformation of Freeman and Tukey were applied when necessary. Tests were two-sided.

Summary of doses received during the study:

Concentration of metam mg/ml	0.01		0.03		0.1	
F0 parent	M	F	M	F	M	F
Week 1-10 mg/kg bw/d	1.7	2.4	4.6	6.2	14	16.9
Pregnancy period mean		2.8		6.9		17.6
Lactation period mean		3.4		9.2		30.4
Mean		2.86		7.3		21.6
F1 parent						
Week 1-10 mg/kg bw/d	1.4	2.0	4.0	4.9	12.3	14.4
Pregnancy period mean		2.0		5.7		14.4
Lactation period mean		2.9		9.6		30.0
Mean		2.3		6.7		19.6

The study is accepted.

B.6.6.1.2a Supplementary studies (Annex IIA 5.6.1)

Data from the open literature:

- Blockade of ovulation in the rat by the fungicide metam sodium: relationship between effects on the Luteinizing Hormone surge and alterations in hypothalamic catecholamine (Goldman et al., 1994)

Findings:

According to the authors, ovariectomized, steroid-primed Long-Evans rats showed a dose-related (25–100 mg/kg, IP) suppression of the surge and a drop in nor epinephrine (NE) when metam was given at 11 h, a few h prior to the expected LH rise. The surge effect was reversed by the α -adrenergic agonist clonidine. With cycling rats, a decline with dose (50–300 mg/kg, 13 h, proestrus) was seen in the percentage of ovulating females, with earlier injections (9 h) being less effective at the highest dose. At all doses, low circulating levels of LH and prolactin at 16 h suggested either a blockade in the proestrus surges of each hormone or a displacement in their time of occurrence. Anterior and posterior hypothalamic NE fell by 3 h post injection and was accompanied by a rise in dopamine, while serotonin was unchanged. Although there was a distinct parallel between the alterations in regional catecholamine (CA) and the incidence of ovulation at the high doses of metam, the relationship did not hold as the dose decreased. A similar dissociation between ovulation and CA was seen when equimolar doses of metam or methylisothiocyanate, a principal metabolite, were given by gavage. At the regional level of analysis employed, the data indicate that while IP injections of metam sodium are able to block the LH surge and ovulation in these rats, the dose-response relationship suggests that, along with induced alterations in CA metabolism, an additional factor may be involved in the observed effects.

RMS comments: in this study, signs of systemic toxicity are not reported and it is difficult to separate primary toxic effects from secondary effects related to systemic toxicity consecutive to the high doses used. The endpoints investigated in this study were not examined in the study provided by the company according to the ongoing guidelines and it is therefore difficult to exclude such an effect. However, RMS concluded that this study does not raise concern, as the doses used in this study are much higher.

Material and methods: metam sodium (95% purity, Chem. services) was given ip to Long-Evans rats after suspension in 4% methylcellulose. Bilateral ovariectomies were performed and animals received a capsule containing estradiol benzoate implanted subcutaneously in order to maintain blood estradiol levels as those reported in intact proestrus rats. Rats were given single injections of metam sodium at 0, 25, 50 or 100 mg/kg at 11 h prior to the anticipated LH rise and blood was collected frequently before sacrifice. In a second study, intact rats were used and were injected metam sodium at 0, 50, 100, 200 or 300 mg/kg. In a third experiment, proestrus rats were injected with metam sodium at 0, 50, 100, 200 or 300 mg/kg and changes in brain catecholamine were studied.

B.6.6.2.1a Teratogenicity test by the oral route in the rat (Annex IIA 5.6.2)

- **Report on the study of the prenatal toxicity of metam-sodium in rats after oral administration (gavage) (Hellwig and Hildebrand, 1987) Historical Control Skeletal Variations in the Metam Sodium Rat Teratology Study (Rose,1991)**

Findings: table B.6.6.2.1-1a:

Mortality did not occur.

Body weight and bw gain: at top dose, a reduction is reported during both treatment and post treatment. BW gains of these animals were also diminished, particularly during the first days of exposure. At 40 mg/kg bw/d, reduced BW gains were observed from day 6-13. When correcting BW gain (bw on day 20- bw on day 0 - uterus weight) show a significant substance related decreased at top dose.

Clinical signs: general behavior was not disturbed.

Food consumption: was reduced in all dams of groups 40 or 120 mg/kg bw/d during exposure and after exposure only at top dose.

Water consumption: no compound-related effect.

Conception rate: In consequence of the significantly increased post-implantation loss in groups 10 and 120 mg/kg bw/d, the number of live fetuses was correspondingly reduced. This effect is not regarded as substance related because there is no dose-response relationship recognizable.

Conception rate, n° corpora lutea and total implantations were not affected by treatment.

Fetus weight: at 120 mg/kg bw/d, mean fetal weight was significantly lower.

Sex distribution was not modified.

Table B.6.6.2.1-1a: developmental rat study after exposure to metam sodium.

Endpoints/dose (mg/kg bw/d)	0	10	40	120
N° not pregnant	1	1	1	3
Body weight:				
Bw Day 8-20:			↓1-3%	↓3-6%*
Bw end study- uterus				↓5%*
Bw end study – beginning – uterus				↓22%*
Bw gain d 6-8		↓22%	↓100%*	↓100%*
D 8-10		↓3%	↓10%	↓30%*
D 10-13		↓11%	↓17%	↓16%*
Food consumption:			↓16-8%	↓20-10%
Placental weight:			↓6%*	↓8%*
Corpora lutea /female	15.21	15.63	14.5	15.05
Total implantation/female	13.75	13.71	12.5	13.9
N° live fetuses/litters	303/24	282/23	277/24	261/22
% per female	93	82*	93	85*
% dead implant/rat (post implantation loss)	7.28	17.9*	6.6	14.8*
Preimplantation loss	10.37	10.20	15.24	7.37
Fetal weight:			↓3%	↓8%*
Total anomalies: n° affected fetuses/n° affected litter (% fetuses /% litter)	34/17 (18.5/70.8)	44/17 (22.5/74)	54*/21 (29.8/87.5)	37/17 (21.8/77.3)
Total variations: n° affected fetuses/n° affected litter(% fetuses /% litter)	90/23 (44/95)	97/22 (50/95)	102*/24 (57/100)	116*/22 (67/100)
Total retardations° affected fetuses/n° affected litter(% fetuses /% litter)	152/24 (75.8/100)	144/23 (77/100)	165*/24 (90/100)	158*/22 (92/100)

*Statistically significantly different from control

Table B.6.6.2.1-2a:

Macroscopic fetus examination: Two fetuses/1 litter at top dose exhibited a meningocele (neural tube closure defect) which is a rare anomaly.

Organ examination after evisceration: a bilateral microphthalmia was reported in one fetus at top dose. Enlarged renal pelvis could be detected in any group including control. This is a common finding in the strain of rats used in this study.

An organ examination was performed according to the method of Barrow/Taylor and the kind of variations revealed again enlarged renal pelvis and hydroureter without compound-related effect.

Skeletal examination:

Anomalies were detected in the vertebral column and in sternum. The number affected litters were increased as compared to the control and sometimes higher at 40 mg/kg bw/d than at 120 mg/kg bw/d.

As regards skeletal anomalies, there was no dose response relationship and/or incidences seen within the historical control range for sternbrae ossification (centers dislocated, ventral segments ribs asymmetrically fused with sternum), thoracic vertebra body dumbbell-shaped (notch of cartilage, cranial) and thoracic vertebra body dumbbell shaped (notches cartilage cranial/caudal).

Variations were detected in sternum and vertebral column. The incidence of affected litters showed a dose-related effect for thoracic vertebra bodies which presented dumbbell-shaped.

This finding, although evaluated as a variation, can be assessed as a retardation as is done by other investigators. Therefore, the increase in compound-related skeletal variations is assessed as another sign of immaturity of fetuses.

With respect to the incidences noted in skeletal variations in the different treatment groups, the observation of thoracic vertebra bodies dumbbell shaped (cartilage unchanged) showed a dose-response relationship, however, the historical control data demonstrated that the incidence in the low dose group of 10 mg/kg bw/day was fully within the historical background range while the concurrent control incidence was comparable to the mean historical value. Thus, effects on the incidences of skeletal variations were evident at the mid and high dose levels, while incidences at the low dose level are covered by historical controls.

NOAEL for skeletal variations is derived at 10 mg/kg bw/day.

Retardations: incomplete or missing ossification of sternbrae, cranial bones, metacarpal/metatarsal bones and pelvic girdle were found at the different dose levels showing sometimes a clear dose-effect relationship, which, at 40 and 120 mg/kg bw/d correspond with the decrease in fetal body weight. These effects are considered a compound-induced sign of fetal immaturity. Incomplete ossification of skull bones was not present in historical control data.

It is possible that the retarded ossification of fetal skeletons, especially the incomplete ossification of skull bones is connected with the mechanism of development of meningocele and influence of metam sodium cannot be excluded.

For the skeletal retardation:

- Interparietal bone incomplete ossification (cartilage present), there were no control data, but as there was no coherent dose-related effect and a direct relationship to treatment could not be established at 10 mg/kg bw/day.
- Sternbrae not ossified (cartilage present) and metacarpal incomplete ossification (bilateral, cartilage present) the incidence at the low dose level of 10 mg/kg bw/day was clearly within the historical control range.
- Metacarpal incomplete ossification (unilateral, cartilage present), there were no control data available. However, the relatively small increase over concurrent controls at the low dose level of 10 mg/kg bw/day was not considered to be of any biological significance.
- Metatarsal incomplete ossification (bilateral, cartilage present) all the study values were outside the historical control range but the relatively small increase in the incidence at 10 mg/kg bw/day was not considered to be of any biological significance.
- Metatarsal incomplete ossification (unilateral, cartilage present), there were no control data available but the incidence of one affected fetus at 10 mg/kg bw/day cannot be considered to be significant as such an incidence of only one affected fetus could have been a change finding.

There were no effects at the low dose level of 10 mg/kg bw/day with respect to generalized retardations of the skeleton.

Historical control data are included in the table B.6.6.2.1-3a.

Table B.6.6.2.1-2a: developmental rat study after exposure to metam sodium.

Endpoints/dose (mg/kg bw/d)	0	10	40	120
Number fetuses/litters	303/24	282/23	277/24	261/22
N° affected fetuses/n° affected litters				
Caesarean section				
Anomalies : Meningocele	0/0	0/0	0/0	2/1
Variations	0/0	0/0	0/0	0/0
Retardations	0/0	0/0	0/0	0/0
Evisceration				
Anomalies : Microphthalmia bilateral				1/1
Variations				
Enlarged renal pelvis bilateral	3/3	11/7	29/13	14/9
Enlarged renal pelvis unilateral	12/6	17/10	43/19	12/8
Retardations	0/0	0/0	0/0	0/0
Barrow Taylor				
Anomalies	0/0	0/0	0/0	0/0
Variations				
Enlarged renal pelvis bilateral	23/13	14/8	5/3	4/4
Enlarged renal pelvis unilateral	17/14	17/12	8/5	14/11
Hydroureter bilateral	3/3	2/2	0/0	0/0
Hydroureter unilateral	3/3	3/3	1/1	2/2
Retardations	0/0	0/0	0/0	0/0
Skeletal findings				
Anomalies				
Vertebral column thoracic vertebra body dumbbell-shaped, notch of cartilage, cranial	9/8	14**/10	17**/13	9/9
Vertebral column thoracic vertebra body dumbbell shaped, notches cartilage cranial/ caudal	21/12	23/11	24/14	20/15
Sternum sternbrae ossification centers dislocated, ventral segments ribs asymmetrically fused with sternum	0/0	2/2	12/11	3/3
Variations				
Vertebral column: thoracic vertebra bodies dumbbell shaped- cartilage unchanged	17/8	22/12	49**/19	62**/21
Vertebral column: thoracic vertebra body dumbbell-shaped	27/14	15/11	28/16	30/17
Sternum xiphoid process bipartited	9/7	16/11	19/11	8/7
Retardations :				
Generalized retard skeleton	0/0	0/0	6/3	7/6
Hindlimb metatarsal bone incomplete ossification	2/2	4/2	11/7	20/10
Forelimb Metacarpal bone incomplete ossification	0/0	1/1	6/4	13/8
Pelvic girdle Pubic bone incomplete ossification	0/0	0/0	0/0	3/3
Sternum:				
sternbra incomplete ossification	72/22	75/23	74/21	52/19
sternbrae incomplete ossification	42/18	25/15	57/21	78/21
sternbra not ossified	34/16	44/17	34/16	30/16
sternbrae not ossified, cartilage present	12/5	7/6	10/8	35**/13
xiphoid process-center bipartited	38/18	25/15	15/14	20/11
Head:				
hyoid bone not ossified	2/1	0/0	2/2	5/4
hyoid bone incomplete ossification	15/12	9/6	13/12	27/11
Interparietal bone incomplete ossification, cartilage present	1/1	8/4	20/11	15/10
Parietal bone incomplete ossification	1/1	8/4	7/3	4/3
Limbs:				
Metacarpal incomplete ossification bilateral, cartilage present	0	1/1	6**/4	13**/8
Metacarpal incomplete ossification unilateral, cartilage present	0/0	3/3	7/5	14/11
Metatarsal incomplete ossification bilateral, cartilage present	2/2	4**/2	11**/7	20**/10

Endpoints/dose (mg/kg bw/d)	0	10	40	120
Metatarsal incomplete ossification unilateral, cartilage present	0/0	1/1	8/5	12/9

** Percentage affected fetuses slightly outside historical laboratory data: see Table B.6.6.2.1-3a.

Table B.6.6.2.1-3a: Historical data on fetuses reported by P. Rose (1991) (litter incidence is not reported)

Type of observation	Organ	Historical mean % - range
Anomalies:	Sternum sternbrae ossification (centers dislocated, ventral segments ribs asymmetrically fused with sternum)	0.9 0-2.2
	Vertebral column thoracic vertebra body dumbbell-shaped, notch of cartilage, cranial	2.1 0.5-4.7
	Vertebral column thoracic vertebra body dumbbell shaped, notches of cartilage cranial/ caudal	2.1 0.5-4.7
Variations:	Vertebral column: thoracic vertebra bodies dumbbell shaped- cartilage unchanged	8.7 2-14.8
Retardations:	Head: Interparietal bone incomplete ossification cartilage present	Not recorded in historical data
	Sternum: sternbrae not ossified, cartilage present	8.7 4.7-16
	Metacarpal incomplete ossification bilateral, cartilage present	0.07 0-0.7
	Metacarpal incomplete ossification unilateral, cartilage present	Not recorded in historical data
	Metatarsal incomplete ossification bilateral, cartilage present	0.1 0-0.7
	Metatarsal incomplete ossification unilateral, cartilage present	Not recorded in historical data
	Generalized retard skeleton	Not recorded in historical data

Conclusion:

Metam sodium caused clear signs of maternal and fetotoxicity at 40 and 120 mg/kg bw/d. A low incidence of a rare anomaly (meningocele) was reported at top dose, which might be connected with the retarded ossification of fetal skeletons, especially incomplete ossification of skull bones.

NOAEL maternal toxicity = 10 mg/kg bw/d taking into account the decreased body weight, body weight gain and food consumption reported at 40 mg/kg bw/d onwards.

NOAEL developmental toxicity = 10 mg/kg bw/d taking into account the dose-related increased incidence of variations reported at 40 mg/kg bw/d onwards.

GLP status: yes (no attest of competent authority)

Experimental protocol not fully in agreement with Directive 87/302/CEE Annex V B or OECD test guideline 414 (2001-1981).

Deviation from official protocol: skeletal examination was performed on 2/3 of fetuses, for visceral examination on 1/3 of fetuses and not vice versa. Clinical signs of mothers not clearly reported.

Material and methods: 25 female Wistar rats/dose group received by gavage an aqueous solution of metam sodium (batch n° ZH 130585; 42.2% or 517.3 g/L) at 10, 40, 120 mg/kg bw/d daily on day 6 through 15 post coitum. Controls received water. The test substance solutions were freshly prepared each day just before treatment.

The study is accepted.

- **Metam sodium: dose range finding study in pregnant rat and developmental toxicity study in the rat (Tinston, 1993)**

Range finding study at 20, 40, and 80 mg/kg bw/d: Table B.6.6.2.1-2a

Findings:

Parental data:

Mortality: All animals survived the scheduled termination.

Clinical signs: no compound-related findings.

Body weight and bw gain:

At top dose of 80 mg/kg bw/d, body weight and bw gain was affected during the gestation period. A less marked effect was seen at 40 and 20 mg/kg bw/d

Macroscopic evidence of pathological changes seen at autopsy (raised areas in non-glandular region of stomach) at top dose is consistent with an irritant effect of metam sodium.

Reproduction parameters were not affected. No effects were seen on number resorptions, implantation, and number fetuses born live.

Fetal data: fetotoxicity was evident at 80 mg/kg bw/d with a reduced mean fetal weight but with no effects on the incidence of intra uterine deaths. At 20 and 40 mg/kg, bw/d there was also some evidence for fetotoxicity as shown by slightly reduced mean fetal weight.

A major defect, a meningocele, was detected in a single fetus at top dose.

Doses of 5, 20 and 60 mg/kg bw/d were selected for developmental toxicity.

Table B.6.6.2.1-2a: developmental rat study after exposure to metam sodium.

Endpoints/dose (mg/kg bw/d)	0	20	40	80
N° pregnant	10/10	10/10	10/10	10/10
Body weight:				
D 16		↓4%	↓6%	↓8%*
D 22		↓5%	↓5%	↓5%
Bw gain d 7-16		↓38%*	↓48%*	↓66%*
Food consumption		↓14%*	↓16%*	↓25%*
Maternal macroscopy: raised areas non glandular stomach		0	0	3 dams
fetal weight:		↓8%*	↓8%*	↓14%*
Fetal examination:				
Meningocele				1 fetus
Malrotated hindlimb	1 fetus			

*Statistically significantly different from control

Conclusion:

Maternal toxicity was seen at all dose levels of metam sodium as shown by reductions in body weight gain and food consumption. Mean fetal weight was reduced at all dose levels. NOAEL maternal toxicity and developmental toxicity < 20 mg/kg bw/d.

Main study at 5, 20, and 60 mg/kg bw/d:

Findings:

Maternal data: table B.6.6.2.1-3a

Mortality: all rats survived to scheduled termination.

Clinical signs: were noted at 20 and 60 mg/kg bw/d. Vaginal bleeding was not associated with fetal resorption except at top dose. At 5 mg/kg, bleeding was reported prior to the start of dosing and was therefore not related to treatment. One female at 60 mg resorbed her litter.

Body weight: a dose-related reduction was seen during the dosing period such that body weight at the end of this period was reduced.

Food consumption: there was a dose related decrease at all dose levels.

Necropsy findings in mothers: renal pelvic dilatation of the kidney was seen at top dose.

A dose-related decrease in percentage pre-implantation loss was seen at all dose levels with a consequent slight increase in mean number of implantations and live fetuses. Since implantation occurs prior to the first day of dosing, these changes are not related to treatment. Post-implantation loss, early and late intra-uterine deaths were not affected.

Table B.6.6.2.1-3a: developmental rat study after exposure to metam sodium.

Endpoints/dose (mg/kg bw/d)	0	5	20	60
N° not pregnant/n° mated	0/24	0/24	1/24	2/24
Clinical signs: vaginal bleeding	0	1	3	3
Piloerection				10
Urinary incontinence			1	10
Stained mouth			5	23
Stained around nose		1	3	9
Body weight:				
D 8-16		↓1-2%*	↓2-4%*	↓5-10%*
D 22		↓1%	↓3%*	↓8%*
Food consumption day 7-16:		↓6%*	↓16%*	↓25%*
Day 19-22:		↓2%	↓5%*	↓11%*
Maternal macroscopy: pelvic dilatation kidney		0	1	4
Litter data:				
Totally resorbed litter at termination	0/24	0/24	0/24	1/24
Live litters in utero at termination	24/24	24/24	23/24	21/24
Preimplantation loss %	20.4	16.8	13.9	10.4*
Post implantation loss %	6.8	7.6	5.9	8.7
N° implantation:	11.6	12.2	12.7	13.5*
Live fetuses	10.9	11.4	12	12.4
fetal weight:			(↓8%*)	↓14%*
Litter weight (g):	54.8	56.6	57.1	53.8
Gravid uterus (g):	78.1	80.6	82	78.6

Litter data (table B.6.6.2.1-3a and 4a):

A dose related statistically significant reduction in mean fetal weight was seen at 20 and 60 mg/kg. Mean litter weights and gravid uterus weights were however similar to controls at mid dose reflecting the slightly increased means number of live fetuses.

Major defects: Incidence was increased at top dose affecting five fetuses, which were no littermates.

A) *External visceral defects:* With one exception (cervical arch), defects affected the head with some fetuses having more than one defect.

The eyes were affected in three of the fetuses, which had other head defects at top dose. The incidence of microphthalmia in one fetus of control group was not accompanied by other major effects.

One fetus had a shortened jaw and cleft lip, one had meningocele.

One fetus at 20 mg/kg had four major defects, three of which were in head and included a shortened lower jaw, which has not been seen in historical data.

The incidence of specific major defects and number of fetuses affected at five and 20 mg was small, not statistically significant, and did not form part of any dose-response relationship, although the possibility of a treatment-related effect cannot be completely dismissed. The authors of the study consider that the findings at 5 and 20 mg/kg are unlikely to be due to treatment with metam sodium.

B) *Skeletal defects:* at top dose, one fetus had unossified second, third and fourth arches of the cervical vertebrae, one fetus displayed an abnormal zygomatic arch, none of which has been seen historically in controls in the laboratory. Two fetuses in the 5 mg/kg group had abnormal zygomatic arch. Whilst this defect was not seen at 20 mg/kg, a single incidence was seen at 60 mg/kg.

Minor defects:

A) *external visceral defects:* no specific findings.

B) *Skeletal defects:* The percentage of fetuses with skeletal defects was statistically increased at top dose. A statistically significant increase in the number of fetuses with unossified fourth, fifth or sixth cervical centrum or with unossified fifth sternebra. Since these areas of the fetal skeleton are amongst the last to ossify at this stage of gestation, the increase in unossified centers is indicative of retarded ossification and fetotoxicity. Minor skeletal defects were not increased at 5 mg/kg bw/d.

Variants: were limited to skeletal findings. Differences from control fetal incidences for unossified odontoid, unossified second cervical centrum or unossified calcaneum were statistically significantly increased for all groups except for the incidence of fetuses with unossified odontoid in the low dose group.

However, the incidence for unossified odontoid and calcaneum at 5 and 20 mg/kg bw/d were within the range seen historically in controls and were considered by the authors of the study to be of no toxicological significance (Table B.6.6.2.1-5a).

The incidence of fetuses with unossified third centrum or partially ossified fifth sternebra and/or unossified ventral tubercle was increased at top dose only. The n° of fetuses with unossified ventral tubercle was increased in treated groups and although the increase was not clearly dose-related, there were statistical differences for the 20 and 60 mg/kg bw/d groups. These changes are consistent with retarded ossification.

A decrease in n° of fetuses with partially ossified seventh cervical transverse processes was seen in the 20 and 60 mg/kg bw/d and this is indicative of retarded ossification.

Manus and *pes* scores indicated there was delayed ossification in fetal hand and foot bones at 60 mg/kg bw/d, suggesting developmental immaturity.

The minor skeletal defects and variants were mainly associated with reduced ossification and the findings are consistent with the reduced fetal weights seen at 20 and 60 mg/kg bw/d.

Table B.6.6.2.1-4a: Foetal/litter examination (Tinston, 1993)

Endpoints/dose (mg/kg bw/d)	0	5	20	60
N° Foetuses / litters	261/24	273/24	276/23	261/21
Major defects:				
a) External/visceral defects:				
Microphthalmia:	1/1			2/2
Anophthalmia:				1/1
Gross malformation skull, upper jaw short, cleft lip				1/1
Lower jaw shortened			1/1	
Meningocele				1/1
Internal hydrocephaly				3/3
<i>Total</i>	1/1	0/0	1/1	4/4
b) Skeletal defects:				
skull Abnormal zygomatic arch:	0/0	2/2	0/0	1/1
Gross malformation			1/1	1/1
Cervical vertebrae 2 nd , 3 rd and 4 th left: arch not ossified:				1/1
<i>Total</i>	0/0	2/2	1/1	3/3
Minor defects:				
Skull sutural bone between interparietal and parietal	1/1	0	0	2/2
Cervical vertebra centrum unossified:				
4 th	4/3	5/3	8/7	27*/13*
5 th	2/2	3/2	3/2	14*/8*
6 th	1/1	0/0	2/2	9*/5
Sternebrae: 5 th not ossified	2/2	2/2	8/5	10*/9*
<i>Total minor skeletal defects</i>	30/16	45/18	47/21	88**/19
Variants :				
Cervical vertebra centrum unossified				
2 nd	44/17	68*/19	109*/20	185*/21*
%	17/71	25/79	39/83	71/88
3 rd	10/6	12/6	18/13	60*/18*
Cervical vertebra unossified ventral tubercle	10/6	12/9	26*/9	22*/13*
Cervical vertebra transverse process partially ossified, 7 th	30/15	31/15	17*/9	15*/7
Sternebrae: partially ossified 5 th	76/20	70/21	80/22	107*/20
Odontoid: unossified	24/13	35/14	56*/18	118*/21*
Calcaneum: unossified	113/22	155*/21	186*/22	245*/21
%	43/92	57/88	67/92	94/88
Mean manus score/litter:	4.21	4.16	4.24	4.54*
Mean pes score/litter:	4.87	4.86	4.91	5.00*
<i>Total skeletal variants</i>	182/24	205/24	235**/23	257**/21

Incidences expressed as n° affected fetuses/ n° affected litters; *, ** Statistically significantly different from control;
Yellow highlighted: considered biologically relevant and/or not covered or HCD not provided

Table B.6.6.2.1-5a: historical incidence of defects in the laboratory from June 1990 up to February 1993.

Endpoint	Fetal incidence- %		Litter incidence -%	
Anophthalmia	3/2577	0.0-0.4%	3/228	0.0-4.5%
Microphthalmia	4/ 2577	0.0-0.4%	4/ 228	0.0-4.5%
Internal hydrocephaly	4/2577	0.0-0.7%	4/228	0.0-8.7%
Skull gross malformation	2/2577	0.0-0.4%	2/228	0.0-4.5%
Calcaneum not ossified	1516/2577	39-82%	217/228	87.5-100%
Cervical vertebrae centrum not ossified 2 nd	790/2577	17.1-46.1%	194/228	74-95.5%

Conclusion:

Administration of 20 or 60 mg/kg bw/d metam sodium was associated with maternal toxicity manifested as a dose-related reduction in body weight gain and food consumption during the dosing period. Clinical signs such as piloerection, salivation and urinary incontinence were treatment related.

Fetotoxicity was evident at 20 and 60 mg/kg bw/d. Reduced ossification were consistent with the reduced fetal weight at 20 and 60 mg/kg bw/d. Major defects were observed essentially at top dose but affected one or two fetuses at the lower dose levels.

Initially, the company proposes to set an overall NOAEL = 5 mg/kg bw/d considering that the incidence of specific major defects and number of fetuses affected at 5 and 20 mg/kg was small, not statistically significant, and did not form part of any coherent dose-response relationship, although the possibility of a treatment-related effect cannot be completely dismissed. However, the company re-assessed the fetal effects and the only effect noted at the mid dose level was a statistical significant reduction in fetal weight. The litter weight was not affected but the litter size was greater at mid dose level and it is believed that the statistically significantly lower fetal body weight at mid dose level is a direct effect of the increased litter size and is not to be considered as a direct effect of treatment. A NOAEL developmental toxicity = 20 mg/kg bw/d is proposed.

RMS considers that considering the litter incidence, some minor skeletal variants are increased at 20 mg/kg bw/d onwards (i.e. third cervical vertebra centrum unossified, cervical vertebra ventral tubercle unossified, and odontoid unossified).

NOAEL parental toxicity = 5 mg/kg bw/d.

NOAEL developmental toxicity = 5 mg/kg bw/d.

Already Peer Reviewed, EFSA 2008

RMS: It should be kept in mind that the incidence of a number of variations in the rat was dose-dependently increased, indicating an involvement of the treatment, at least at 20 mkd and higher. In addition, the increase of a number of variants was not covered by historical control data. The variants were all related to partial or complete ossification failures, which were moreover replicated in the other rat developmental study. While ossification delays generally not merit a classification when considered in isolation, they should be considered for the establishment of the foetal toxicity/developmental NOAEL. Such findings are moreover particularly relevant for a compound, which is able to induce also more serious anomalies (albeit in the presence of maternal toxicity).

Therefore the next-lower developmental NOAEL value of 5 mkd was maintained by the RMS. The parental NOAEL was also 5 mkd. This was agreed upon during the PRAPeR meeting.

GLP status: yes (no attest of competent authority)

Experimental protocol fully in agreement with Directive 87/302/CEE Annex V B or OECD test guideline 414 (2001-1981).

Material and methods:

Range finding study:

Groups of 10 time-mated female Alpk: APfSD (Wistar derived) rats/group received by gavage 0, 20, 40 or 80 mg/kg bw/d metam sodium (ref n° BAS/005/00N, CTL n° Y06930/008; 43.15% w/w) in water. The rats were dosed on day 7-16 inclusive of gestation and were killed on day 22. One preparation of dosing formulation was made up at two time points and used for a maximum period of 7 days and stability was reported for this period. The achieved concentrations were within 3% of nominal

Main study: 24 time-mated females rats Alpk: APfSD (wistar derived)/ group received by gavage 0, 5, 20 or 60 mg/kg bw/d metam sodium (ref n° BAS/005/00N, CTL n° Y06930/008; 43.15% w/w) in water. Control rats received water only. The rats were dosed on days 7-16 inclusive of gestation and were killed on day 22. Samples of formulation were analyzed prior to the start of the dosing in order to determine the stability of metam sodium in water. The concentrations for each preparation were within 10% of the nominal concentration. Overall, the mean concentrations were within 2% of the nominal concentration. The dosing solutions were used for no longer than 5 days from the date of preparation.

Statistics: covariance analysis of body weight; analysis of variance for food consumption, number of implantations, uterus weight, litter weight, fetal weight. Maternal performance data and proportion of litters with each defect were considered by Fisher's exact test. All statistical tests were two-sided.
The study is accepted.

B.6.6.2.2a Teratogenicity test by the oral route in the rabbit (Annex IIA 5.6.2)

- **Metam sodium: developmental toxicity study in the rabbit and Dose Range Finding Study In The Pregnant Rabbit (Hodge, 1993)**

Findings:

Range finding study was performed with two rabbits receiving 80 mg/kg bw/d during 10 days:

Apart from appearing slightly subdued on day 3, the rabbits were clinically normal. However, food consumption was decreased between day 1 and 4 and lost substantial amount of weight up to day 4 or 5 respectively. The animals then began eating and gained weight although they did not regain their initial body weight. Fecal output was decreased throughout most of the dosing period.
These data suggest that 80 mg/kg bw/d exceeded the MTD.

Range-finding study at 0, 5, 20, 40, or 60 mg/kg bw/d (Table B.6.6.2.2-1a)

The highest dose tested, 60 mg/kg bw/d produced clear signs of maternal toxicity manifested by reduced body weight gain and food consumption. Clear fetotoxicity was identified by increased post implantation loss and reduced fetal weight. This dose level is considered the MTD for fetal viability. Slight maternal toxicity was noted at 40 mg/kg bw/d and slight evidence of fetotoxicity was evidenced. At 20 mg/kg bw/d, an increased incidence of few feces was noted in dams. This is considered not to be an adverse reaction.

Table B.6.6.2.2-1a: range-finding study with metam sodium in rabbits.

Endpoint/dose	0	5	20	40	60mg/kg bw/d
Clinical signs:					
Few feces				Increased incidence	
Bw gain d 8-20				↓56%	↓61%*
Food consumption:				↓38%*	↓46%*
Litter data:					
Resorption				2	
Gravid uterus weight				↓21%	↓33%*
Litter weight				↓26%	↓40%*
Fetal weight				↓6%	↓11%
Early intra-uterine death	3	3	6	8	27*
N° fetuses/n° litters	64/8	56/6	65/7	32/5	44/8
Post implantation loss%	5.9	13.8	8.5	22	41.3
Abnormalities:					
Cyclopia					1/1
Spina bifida meningocele	1/1	1/1			

*Statistically significantly different from control

Main study at 5, 20, or 60 mg/kg bw/d:

Maternal data: table B.6.6.2.2-2a.

Mortality: Two rabbits were killed on day 24 for human reasons (20 mg/kg bw/d) and following abortion (60 mg/kg bw/d). At 5 mg/kg bw/d, there were no specific clinical signs in an animal found dead.

Clinical signs: at top dose, there was an increase of few feces and red/orange staining on the cage tray.

Abortion, which is a common response in the New Zealand White rabbit, was not increased.

Maternal body weight: at 60 mg/kg bw/d, there was a considerable weight loss after starting of dosing. Weight gain from the start of dosing onwards was clearly reduced throughout the study. Similar but less marked changes were seen in the 20 mg/kg bw/d group.

Food consumption: was reduced at top dose particularly on days 8-11. Some evidence of increased food consumption during days 23-30 was reported and this was most obvious in the animals, which totally resorbed their litters. At 20 mg/kg, there was a slight effect.

Table B.6.6.2.2-2a: mean developmental metam sodium study in rabbits.

Endpoint/dose (mg/kg bw/d)	0	5	20	60
Mortality		1 day 26	1 day 24	1 day 24
Not pregnant	0/20	3/20	2/20	1/20
Total resorption at termination				9/20*
Live fetuses in utero at termination	20/20	16/20	17/20	9/20*
Body weight				
Day 9-20			↓1-2%	↓2-5%*
Food consumption: d8-20			↓19-17%*	↓70-29%*
Litter data:				
Gravid uterus weight				↓31%*
Litter weight				↓27%*
Fetal weight			↓4%	↓14%*
Pre implantation loss:				
proportion affected implants/total n° implants	49/238	20/185*	24/183	12/99
Proportion litters affected/total n° litters	16/20	8/16	11/17	6/9
Post implantation loss:				
proportion affected implants/total n° implants	21/189	17/165	11/159	35/87*
Proportion litters affected/total n° litters	10/20	9/16	6/17	9/9*
Early intrauterine death:				
proportion affected implants/total n° implants	17/189	11/165	8/159	100/156*
Proportion litters affected/total n° litters	8/20	8/16	6/17	18/18*
Proportion male fetuses	72/168	79/148	86/148*	21/51*
Mean n° live fetuses	8.4	9.25	8.71	2.89*

*Statistically significantly different from control.

Litter data Table B.6.6.2.2-3a:

High level of total resorptions and post-implantation loss at 60 mg/kg was reflected in a decrease in litter size with lower gravid uterus weight and litter weight. Fetal weight was also decreased. Percentage of male fetuses was also reduced and litters containing five or less fetuses did not included males.

No effects were seen at 20 or 5 mg/kg bw/d.

Major defects: these were single incidences, which showed no dose response. Three fetuses with cleft palate were seen, which is an unusual finding in control rabbits in this laboratory. This defect was seen most recently in a control rabbit fetus (a runt) in a study conducted in January 1990. Two of the three affected fetuses in this study were small (<27 g) and one of these and the other of normal size showed multiple defects which included exencephaly in the 5 mg/kg fetus. However, the incidence was higher than expected and while the possibility of cleft palate being induced by metam sodium cannot be totally discounted, overall the cleft palate in this study is considered not to be compound-related.

Minor defects: skeleton was affected in some fetuses and some differences compared with control achieved statistical significance. However, there was no clear dose-related litter incidence.

Variants: the overall incidence in fetal skeletal variants increased at 60 mg/kg.

In fetuses at a dose level of 20 mg/kg bw/day there was a single incidence of one affected foetus in one litter with 7th sternbrae present is considered to be an isolated finding with no toxicological and biological significance.

The increased incidence of 27 pre-sacral vertebrae at 20 mg/kg was within the historical control range.

Furthermore, skeletal variations were noted in 20/20 litters, 16/16 litters, 17/17 litters and 9/9 litters in the control, low, mid and high dose group, respectively, which demonstrated that also in the control group all litters were affected with respect to skeletal variations.

Manus and *pes* assessments were normal.

Table B.6.6.2.2-3a: fetal/litter data.

Endpoint/dose (mg/kg bw/d)	0	5	20	60
affected fetuses /affected litters (n° of fetus)				
Major defects				
External /visceral defects				
Head: exencephaly		1(21)/1		
Meningocele				1(64)/1
Cleft palate		1(21)/1		2(64, 75)/2
Internal hydrocephaly	1(16)			
<i>Spina bifida</i> meningocele	1(20)/1			
<i>Spina bifida</i> rachischisis	1 (20)/1			
Hindlimb malrotated	2	1(21)/1		1(77)
Hindlimb malformed				1(77)
Skull gross malformation				1(64)
Zygomatic arch abnormal	1(16)			
Vertebral column major defect	1(20)			
Sacral vertebra malformed				1(77)
Caudal vertebrae absent				1(77)
Fused /absent ribs			1(56)/1(45)/2	
Pelvic girdle				1(77)
Minor defects				
Skeletal defects:				
Cervical vertebra, transverse process partially ossified 7 th	0/0	3/1	6*/3	1/1
Sternebrae 7 th present	0/0	0/0	1/1	7*/2
Sternebrae 5 th not ossified	22/9	17/6	15/8	0*/0*
Extra rib: 13 th , short length and floating	15/9	23/9	13/8	0*/0*
Variants				
Odontoid partially ossified	75/16	83/15	75/15	42*/9
Vertebral column , 27 pre-sacral vertebrae	34/13	26/9	50*/13	37*/9
Extra rib: 13 th , normal length	60/16	49/11	68/17	45*/9
Sternebrae 5 th partially ossified	64/18	54/14	55/15	7*/5
Total skeletal variations:	20/20	16/16	17/17	9/9

*Statistically significantly different from control.

Conclusion:

The level of maternal toxicity at 60 mg/kg bw/d probably exceeded a MTD and was associated with increased embryonic lethality. The absolute number of major abnormalities was increased at 60 mg/kg bw/d because of the decreased number of viable fetuses. Specific defects included cleft palate which is uncommon in control New Zealand White rabbits in this laboratory.

NOAEL maternal toxicity= 5 mg/kg bw/d based on reductions in food consumption and body weight at 20 mg/kg bw/d.

NOAEL developmental toxicity = 20 mg/kg bw/d based on increased fetotoxicity at 60 mg/kg bw/d.

GLP status: yes (no attest of competent authority)

Experimental protocol: of the main study is in agreement with dir 87/302/EEC Annex VB.

Material and methods:

Range finding study: Two New Zealand white rabbits received 10 daily doses of metam sodium (batch n° BAS/005/00N, CTL n° Y06930/008; 43.14%) by gavage in water at 80 mg/kg bw/d. The dosing solution was prepared daily.

Groups of eight time-mated female New Zealand White rabbits received by gavage 0, 5, 20, 40, or 60 mg/kg bw/d metam sodium (batch n° BAS/005/00N, CTL n° Y06930/008; 43.15%) in water on days 8-20 inclusive of gestation. The day of mating was designed day 1. On day 30 of gestation, rabbits were killed. The formulations were stable for 7 days and were within 8% of nominal values.

Main study: groups of 20 time mated female New Zealand white rabbits were dosed by gavage with 0, 5, 20 or 60 mg/kg bw/d metam sodium (batch n° BAS/005/00N, CTL n° Y06930/008; 43.14%) in water from day 8-20 of gestation (day 1: day of mating). On day 30, rabbits were killed. The dosing formulations were made up at approximately weekly intervals. The formulations were stable for 7 days and were within 8% of nominal values.

Statistics: analysis of covariance for maternal bw; analysis of variance for food consumption, n° implants, live fetuses, gravid uterus weight, litter weight, fetal weight and manus pes scores. Maternal performance data were analyzed by Fisher's exact test. Percentages were analyzed by variance and proportion affected fetuses and litters by Fisher's exact test. All tests were two-sided. The study is accepted.

- **Report on the study of the prenatal toxicity of metam-sodium in rabbits after oral administration (Hellwig, 1987)**

Findings:

Preliminary range-finding study at 50, 100 and 200 mg/kg bw/d:

A preliminary range-finding study was performed on 15 rabbits/dose at 50, 100 and 200 mg/kg bw/d from day 6th to 18 of gestation. The number of dead implantations shows a dose-dependent increase, and there was a marked increase in late abortion at 100 and 200 mg/kg bw/d. live fetuses were decreased. Uterus weight was decreased at 100 and 200 mg/kg bw/d. Body weight of dams at top dose decreased from day 11 of gestation until end of the study. At the end of the study, body weight of dams exposed at 100 mg/kg bw/d was impaired. The doses for the main study were selected based on these results.

Main study at 10, 30 or 100 mg/kg bw/d:

Maternal data: Table B.6.6.2.2-4a

Mortality: there were no cases of spontaneous mortality. One control animal delivered on day 28 and was sacrificed and two does of 10 mg/kg bw/d had to be sacrificed because of fracture of lumbar vertebral column during gavage.

Clinical signs: general behavior was not disturbed in any animals of the study.

Body weight: bw gain at top dose was somewhat lower on most days of treatment but mean body weights of these does were not clearly reduced. The influence on body weight gain partly corresponds with the diminished feed consumption. The results of corrected body weight gain show no dose-related differences between groups.

Necropsy findings: no compound-related findings were reported.

Uterus weight was reduced at top dose.

Reproduction parameters: the conception rate varied between 100 and 93%. Number corpora lutea, total implantations and preimplantation loss were not affected. In test group 30 and 100 mg/kg bw/d, the number of dead implants was increased, especially due to the high number of early resorptions. Increased post implantation loss and reduced number of live fetuses were seen at both dose levels.

Table B.6.6.2.2-4a: mean developmental metam sodium study in rabbits.

Endpoint/dose(mg/kg bw/d)	0	10	30	100
Mortality	1 day 28	2 day 7		
Conception rate %	100	100	93.3	100
Body weight:	No compound-related effect			
Body weight gain				
Day 9-21				↓64-100%
Day 18-21				↓20%
Day 21-29				↓21-67%*
Day 25-29			↓11-56%	
Food consumption: d 7-18				↓7-8%
Corpora lutea number:	103	101	111	117
Total number fetuses/litters :	81/14	85/13	73/14	48/14
placenta weight				↓38%
Fetal weight	No biological significant effect			

Endpoint/dose(mg/kg bw/d)	0	10	30	100
Pre implantation loss:	17.59	8.74	23.79	8.84
Post implantation loss:	3.65	8.06	12.36*	54.93**
Proportion male fetuses	No compound-related effects			
Mean n° dead implants	0.29	0.54	0.79	3.93
Mean n° live fetuses/pregnant rabbit	5.8	6.5	5.2	3.2
% live fetuses/ pregnant female	96.35	91.94	87.64*	45.07**

*: trend; **:statistically significantly different from control.

Litter data: Table B.6.6.2.2-5a:

There were no statistically significant differences between treated and control groups with respect to anomalies, variations and retardations. However, two fetuses in two litters of 100 mg/kg bw/d showed a meningocele or *spina bifida*. These are rare anomalies in the strain of rabbits used in this study.

There was a slight dose-related increase in sternebra asymmetry. The influence of metam sodium cannot be ruled out. The rather low incidence of skeletal retardations in the top dose group may be attributed to the low number of live fetuses, resulting in a greater number of mature fetuses.

Table B.6.6.2.2-5a: fetus/Litter data.

Endpoint/dose(mg/kg bw/d)	0	10	30	100
affected fetuses /affected litters				
Cesarean section :				
Major defects				
Head: Meningocele				1/1
Vertebral column: <i>Spina bifida</i>				1/1
Organs of fetuses:				
Anomalies				
Gall bladder agenesis			1/1	1/1
<i>truncus arteriosus communis:</i>				1/1
Skeletal examination:				
Variations:				
Sternebrae asymmetrical	1/1	2/2	7/3	6/4
Historical data- Himalayan rabbits:	Untreated rabbits			
Agenesis of the gall bladder	1/1			
Asymmetrical sternebrae	-/-			

Conclusion:

Administration of metam sodium to pregnant rabbits during organogenesis elicited clear signs of maternal toxicity at 100 mg/kg bw/d. decreased body weight gain was observed from day 25-29 at 30 mg/kg bw/d. NOAEL maternal toxicity = 10 mg/kg bw/d.

Signs of embryo toxicity such as an increased number of dead implantations and decreased number of live fetuses were reported at 30 mg/kg bw/d. A NOAEL developmental toxicity = 10 mg/kg bw/d is proposed.

Rare defects were observed at top dose.

GLP status: yes (no attest of competent authority)

Experimental protocol: in agreement with OECD guideline 414 (2001-1981).

Material and methods: 15 female Himalayan (Chbb: HM, outbred) rabbits/group received by gavage metam sodium (test substance n° 85/232, batch n° ZH 130585; 42.2%) in water at 10, 30 or 100 mg/kg bw/d from day 6 through 18 post insemination. Controls received water only. On day 29, all females were sacrificed. Stability of metam sodium solution was carried out.

A preliminary range finding study was performed at 50, 100 and 200 mg/kg bw/d.

Statistics:

Dams and fetuses: Williams test was used for analysis of body weight, uterus and placenta. Fisher test was used for conception rate, mortality, percentage litters with anomalous fetuses. Krauch test was used for corpora lutea, implantations, % live and dead and anomaly of fetuses.

The study is accepted.

Already Peer Reviewed, EFSA 2008

Open point: 2.13 The proposed classification as Repro Cat.3 to be discussed in a meeting of experts

[See reporting table 2(31)]

RMS: The most important major and rare anomalies encountered in the various developmental studies can be synthesised as follows (cfr. DAR):

Rat: 2 meningoceles at 120 mkd (also reported at a higher incidence in a RF at 240 mkd) (Hellwig and Hildebrand, 1987), one meningocele at 80 mkd (Tinston, 1993, RF study), 2 microphthalmia, 1 anophthalmia, 1 short upper jaw/cleft lip, 1 meningocele and 3 hydrocephalia at 100 mkd (Tinston, 1993, main study).

Rabbit: 1 cyclopia at 60 mkd (Hodge, 1993, RF), 1 meningocele, 2 cleft palate (Hodge, 1993, main), 1 meningocele/spina bifida (Hellwig, 1987). In addition, increased post-implantation loss was observed at 30, 60 and 100 mkd (Hellwig, 1987, and Hodge, 1993).

The malformations occurred at low incidences (sometimes in singularity), but in a consistent manner, at the top-doses, in the presence of quite severe maternal toxicity. In certain studies, single rare malformations were also present at lower doses, however in these cases, no dose-dependency was demonstrated.

Overall, RMS concluded that the top-dose anomalies fully justified a classification Xn; R63 (Cat. 3), but in the view of the low incidence and the association with severe maternotoxicity, it was felt that a classification in Cat. 2 was indeed not warranted. This was agreed upon during the PRAPeR meeting.

B.6.6b reproduction studies with MITC (Annex IIA 5.6)

B.6.6.1b Two generation reproductive toxicity in the rat (Annex IIA 5.6.1)

- **Technical methyl isothiocyanate (MITC): 2 generation oral (drinking water) reproduction study in the rat (Chemistry report Barker, 1986) and addendum to the report (Jackson, 1988)**

Findings: Table B.6.6.1.1-1b.

P generation:

Parent:

Mortality: no parental rats died.

Clinical signs: there were no abnormalities considered treatment-related.

Body weight:

Some variations in weight gain were observed in both sexes and occasionally attaining statistical significance. Significant reductions in weight gain were observed in females from group 10 and 50 ppm during week 0-7 without relationship with dose. Gestation and lactation weight were not altered.

Food intake was marginally lower than controls for the top dose group without reaching statistical significance. Food conversion efficiency was comparable for all groups.

Water intake: a dose-related reduction in water intake was observed in animals at 10 and 50 ppm during pre-mating period and during lactation period, affecting essentially top dose rats and at a lower level 10-ppm exposed rats.

Mating performance and fertility were not adversely affected.

Necropsy and histopathology findings: no gross or microscopic lesions attributable to treatment were seen in any of the animals examined.

Organ weight: a statistically significant increase in relative pituitary weight was observed in top dose females

Litter data:

F1a pup/litter data: the duration of gestation was within normal limits in all groups. The number of pups born/female was marginally lower at 10 and 50 ppm without reaching statistical significance. In the absence of a dose-response and since control pup mortality values were unusually low, the higher incidences of pup deaths in treated groups were considered unrelated to treatment. Hydrocephaly was observed in four F1 pups, from two different litters in each group 10 and 50 ppm. All of these pups died between day 19 and 21 post-partum. An effect of treatment was considered unlikely as this defect is known to occur spontaneously in this strain of rat at these laboratories and was not observed at these dose levels in the next generation.

Pup weight: there was no effect of treatment.

Organ weight: gonad/body weight ratio was increased in females of 10 and 50-ppm groups. These results are considered as a spurious effect, exacerbated by the technical difficulty in removing and weighing organs of this size.

Table B.6.6.1.1-1b: 2-generation rat study exposed to MITC. Parent P0 data.

Endpoint/dose	0		2		10		50 ppm	
	M	F	M	F	M	F	M	F
intake: mg/kg bw/d	0	0	0.16	0.21	0.76	1.01	3.58	4.76
Body weight gain pre-mating			-	↓ 2%	-	↓ 7%	-	↓ 8%
gestation						↓ 7%		↓ 9%
lactation								↓ 28%
Food intake Premating wk 1-10							↓ 4%	↓ 9%
Water intake pre-mating wk 1-10					↓ 3-12%*	↓ 4-17%	↓ 15-25%*	↓ 7-25%*
Lactation period						↓ 11%*		↓ 20%*
N° P mated females		30/30		28/30		30/30		30/30
Total n° pregnant		30		28		29		27
Fertility index %		100		93.3		96.7		90
Fecundity index %		100		100		96.7		90
Viability index % day1		98%		94.8%		97.2%		98.6%
D 21		93.4%		78.9%		76.5%		85.6%
N° pups born/female		14.6		14.7		13.8		13.7
Pre-weaning loss %		6.6		21		23.5		14.4

* Statistically significantly different from control

F1 generation: Table B.6.6.1.1-2b

Parent:

Mortality and clinical signs were not related to MITC exposure.

Body weight of top dose males was slightly lower during week 14-21. Females were not affected.

Food intake of treated males and females were comparable to controls throughout the pre-mating period. There was no adverse effect of treatment on mean food conversion efficiency.

Water intake was affected in top dose animals during pre-mating but also during lactation.

Mating performance and fertility were not adversely affected.

F1a litter data:

Gestation length was slightly shorter in treated groups but this effect was considered incidental to treatment.

Pup number and viability: treatment did not adversely affect the number of pups born/dam or their viability. The marginally higher incidence of pup deaths in dose 10 and 50 ppm was not statistically significant, was not dose-related and was less than that seen in the F1a litters. It is unlikely that MITC exposure was responsible.

Necropsy findings: hydrocephaly was seen in one group 2-ppm pup, which died on day 1 post-partum.

Pup weight: growth was comparable amongst groups

Physical and functional development of pups was comparable amongst all groups. Slight delays in the onset of eye opening and pinna unfolding were observed at top dose but were considered incidental to treatment, as there were no delays in the completion of each parameter. Functional development of top dose pups was comparable to controls on days 1, 17 and 21.

In the F1 generation adults, the incidence of gross and microscopic findings was low. Macroscopic examination of F2 pups revealed a low incidence of commonly occurring lesions.

Organ weight: no compound-related findings.

Table B.6.6.1.1-2b: 2-generation rat study exposed to MITC. Parent F1 data.

Endpoint/dose	0		2		10		50 ppm	
	M	F	M	F	M	F	M	F
intake:mg/kg bw/d	0	0	0.15	0.19	0.71	0.87	3.4	4.22
Mortality			2					

Endpoint/dose	0		2		10		50 ppm	
	M	F	M	F	M	F	M	F
intake:mg/kg bw/d	0	0	0.15	0.19	0.71	0.87	3.4	4.22
Body weight								
Premating wk 1-10							↓ 4-5%	↓ 3-4%
wk 14-21							↓ 7-8%	
Gestation								↓ 5-7%
Lactation								↓ 6-8%
Food intake premating							↓ 2%	↓ 5%
Water intake premating						↓ 4-24 %*	↓ 17-28%*	↓ 19-37 %*
Lactation period								↓ 19-37 %*
N° F1 mated females		25/25		21/23		24/25		25/25
Total n° pregnant		24		20		21		25
Fertility index %		96		87		84		100
Fecundity index %		96		95.2		87.5		100
N° females surviving to D1 lactation		23		20		20		24
Viability index % day1	97.6%		96.5%		91%		96.6%	
d 21	94%		94%		91.5%		91.3%	
N° pups born/female	11		11.4		12.3		11	
Pre-weaning loss %	8.3		9.3		16.7		11.8	

* Statistically significantly different from control

Conclusion:

Toxicity to adults comprised a reduction in body weight gain during premating, gestation and lactation. The dose-related reduction in water consumption at 10 and 50 ppm reflects the unpalatability of MITC.

NOAEL parental toxicity= 10 ppm (0.76-1.01 mg/kg bw/d).

NOAEL reproduction toxicity > 50 ppm (3.6 mg/kg bw/d).

NOAEL offspring toxicity > 50 ppm (3.6 mg/kg bw/d).

GLP status: yes (no attest of competent authority)

Experimental protocol not fully in agreement with Directive 87/302/CEE Annex V B or OECD test guideline 416 (2001-1983).

Deviation from official protocol: Histopathology was limited to 10 parents of control and top dose group. Histopathology of cervix was not performed. Clinical signs were not reported in a summary.

Material and methods: 30 Charles River CD Sprague –Dawley rats/sex/dose received in their drinking water MITC (batch n° 340178; purity ?) at nominal concentration of 2, 10 or 50 ppm for 70 days before being mated to produce F1a litters which were reared to weaning. 25 offspring from each group were selected at weaning to form F1 generation, which were treated for 77 days prior to pairing to produce F2a litters, which were reared to weaning.

MITC was intended to be at 2.4, 12 and 60 ppm in water. The stock solutions showed some loss of MITC over the storage period. The stock solutions from each week gave values in the range 69% to 95.6% of theoretical values except for weeks 1 and 12 where results at preparation were 37.2 and 66.2% respectively. The working dilutions and cage bottles showed no general loss of MITC i.e. approximately equal losses and gains were shown over the storage period.

The study is accepted.

B.6.6.2.1b Teratogenicity test by the oral route in the rat (Annex IIA 5.6.2)

- **Report on the study of the prenatal toxicity of MITC in rats after oral administration (gavage)(Hellwig and Hildebrand, 1987)**

Findings:

Mortality: there was no spontaneous mortality.

Clinical signs at the end of the treatment, several dams at top dose had sticky and/or moist fur in the area of the snout before, and at the same location reddish but mainly dry fur after gavage.

Body weight and body weight gain were significantly reduced at top dose. A marginal effect was seen at intermediate dose and these effects are attributable to MITC. When body weight gain was corrected (bw day 20- bw day 0- uterus weight), a substance related effects was evident at 10 and 30 mg/kg bw/d. At 3 mg/kg bw/d, corrected bw gain was also slightly, but statistically significantly diminished. This effect was due to 1 dam, which was declared pregnant, but had only really resorptions. It consequently showed reduced or even negative bw gains, especially at the end of the treatment and during the post-treatment periods. This dam is an outlier and the slight decrease in corrected body weight gain in the 3 mg/kg bw/d is regarded as incidental.

Water consumption, which was not measured, but roughly estimated during this study, seemed to be increased in individual dams at 10 and 30 mg/kg bw/d.

Reproduction data: there were no significant and/or dose-response related differences between the groups.

Foetal data:

Foetal weight: The number of foetuses weighing < 75% of the mean fatal weight/litter was increased at top dose.

Placenta weight: was significantly lowered at top dose.

Sex distribution was comparable in the different test groups.

Macroscopic examination of the foetuses:

After evisceration, only variations (enlarged renal pelvis and or bilateral) were observed in any group without any dose-effect relationship. No anomalies or retardations were detected in any group.

In the examination of the organs of the foetuses according to the method of Barrow/Taylor, one anomaly (anophthalmia) was detected in one foetus/one litter at 10 mg/kg bw/d. The kind of variations revealed again was enlarged renal pelvis and hydroureter. No retardations were seen in any group.

Skeletal examination:

There were no statistically significant differences between the treated groups and the controls with regard to anomalies, variations and /or retardations.

Table B.6.6.2.1-1b: developmental toxicity study of MITC in rats.

Endpoint/dose (mg/kg bw/d)	0	3	10	30
Not pregnant		3/25	7/25	2/25
Clinical signs				
Moist fur				9/25
Body weight:				
Day 10-20			(↓1-2%)	↓5-8%*
Body weight gain:				
Day 6				↓13%*
Day 8			↓10%*	
Day 15				↓17%*
Corrected BW gain		↓12%*	↓15%*	↓31%*
Food consumption Day 6-13				↓20-30%
Uterus weight				↓7%*
Placenta weight:				↓6%*
Rate of conception :	100%	88%	72%	92%
N° foetuses /n° litters	300/25	246/21	223/18	262/23
Mean fetal weight g:	3.69	3.79	3.76	3.66
Preimplantation loss	11.5	11.9	10.3	10.9
Mean n° live foetuses	12	11.2	12.4	11.4
% live foetuses/pregnant dam	90.4	82.8	92.4	86.6
Mean n° dead implantations	1.24	1.86	1.11	1.87
% dead implantation/pregnant rat	9.6	17.2	7.6	13.4
Small foetuses n°	1	2	1	5
Organ examination : fetal/litter incidence				
Renal pelvis enlarged uni- or bilateral:				
After evisceration	51/20	23/16	15/10	36/15
After method of Barrow/Taylor	16/8	12/9	12/9	16/11
Anomalies :				

Endpoint/dose (mg/kg bw/d)	0	3	10	30
anophthalmia after method of Barrow/Taylor			1/1	

*Statistically significantly different from control

Conclusion:

NOAEL maternal toxicity = 3 mg/kg bw/d taking into account the reduced body weight gain of dams and an increased water consumption observed at 10 mg/kg bw/d.

NOAEL embryo/fetal toxicity = 10 mg/kg bw/d based on the reduced fetal weight observed at 30 mg/kg bw/d. There were non indications for any teratogenic effects up to the highest dose level.

GLP status: yes (no attest of competent authority)

Experimental protocol fully in agreement with Directive 87/302/CEE Annex V B or OECD test guideline 414 (2001-1981).

Material and methods: 25 female rats Wistar (Chbb=THOM (SPF))/ group received by gavage MITC (batch n° 6205 MK; 96.9%) in olive oil at 3, 10, or 30 mg/kg bw/d from day 6 through day 15 post coitum. Control rats received olive oil alone. Rats were sacrificed on day 20. Stability was guaranteed for the period of the study and was proven before and after termination of the study.

Statistics: Williams test for body weight, uterus and placental weight. Fisher test for conception rate, mortality, and percentage litters with anomalous fetuses and fetuses with anomalies. Krauth test for corpora lutea, implantations, percentage live fetuses and effects on litters. The study is accepted.

B.6.6.2.2b Teratogenicity test by the oral route in the rabbit (Annex II A 5.6.2)

- **Report on the study of the embryotoxicity (including teratogenicity) in the rabbit with MITC (Becker et al. 1986)**

Findings:

Mortality: no death occurred in any dose group and in control except one female at top dose which died due to a supposed injurious intubation.

Clinical signs: no test article related signs or symptoms was observed in any female of the different groups.

Body weight: slight loss was noted from day 6-8 in the treated animals and from day 6-9 in the vehicle control group. At top dose, a slightly higher reduction of mean body weight was considered to be compound-related.

Food consumption: was reduced at top dose from day 6-11.

Reproduction data: no effects were seen on the mean number of implantations, pups or embryonic deaths.

Necropsy findings: no compound related effects were noted.

Litter data:

Body weight of fetuses were not affected.

Sex ratio was similar in all groups.

Investigations of the crania of the fetuses did not show compound-related effects.

Investigation of the body cavities of fetuses did not show a compound related effect.

Skeletal findings: no test article related effects were seen.

Table B.6.6.2.2-1b: developmental MITC study in rabbits.

Endpoint/dose(mg/kg bw/d)	0	1	3	10
Mortality				1
Body weight:				↓5% day 8-26
Bw gain day 6-11				↓3.6%
Litter data: n° examined fetuses	120	134	116	118
Body weight of fetuses mean (g)	31.7	33.7	34.4	32.5
Resorptions	14	21	20	14

Conclusion:

Administration of MITC to pregnant rabbits during organogenesis elicited signs of maternal toxicity at 10 mg/kg bw/d. NOAEL maternal toxicity = 3 mg/kg bw/d. There was no influence of MITC on the investigated reproduction data. A NOAEL developmental toxicity = 10 mg/kg bw/d is proposed. MITC was not embryo/fetotoxic and did not elicit abnormal findings in the litters.

GLP status: yes limited to attest of GLP/QAU

Experimental protocol: in agreement with OECD guideline 414 (2001-1981).

Material and methods: 16 female Chinchilla (Kfm: CHIN, hybrid) rabbits/group received by gavage MITC (batch n° ZNT 85/231-2; 98%) in corn oil at one, 3 or 10 mg/kg bw/d from day 6 through 18 post insemination. Controls received corn oil only. The test article was prepared daily. On day 28, all females were sacrificed. Stability of metam sodium solution was carried out.

A preliminary range finding study was performed at 50, 100 and 200 mg/kg bw/d.

Statistics:

Dams and fetuses: Williams test was used for analysis of body weight, uterus and placenta. Fisher test was used for conception rate, mortality, percentage litters with anomalous fetuses. Krauch test was used for corpora lutea, implantations, percentage live and dead and anomaly of fetuses.

The study is accepted.

WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Registration must not be granted on the basis of this document.

B.6.6.3 Summary of reproductive toxicity and teratogenicity (Annex IIA 5.6)

In rats, metam sodium was tested in a multigenerational drinking water study corresponding to 1.4-216 mg/kg bw/d.

At top dose, body weight was decreased on occasion in males and in females during the different phases of the study. Food consumption was reduced. In females, minimal to marked Bowman's gland duct hypertrophy with loss of alveolar cells was detected in the olfactory mucosa lining the nasal septum and turbinate bones at all levels of the nasal cavity. The changes in the Bowman's gland were accompanied by degeneration/disorganization/atrophy of the olfactory epithelium. The nasal lesion was a direct consequence of the increased dose taken by females during lactation. Nasal passage of offspring of either generation at any dose level was not affected.

The percentage of pups live born was in excess of 94% for each group in both generations. There were no litter losses. There was a reduction in individual pup weight and in total litter weight and a reduction of pup weight gain at top dose in both generations. One female pup of top dose had no ocular issues. Bilateral anophthalmia seems to occur spontaneously in the Alpk: APfSD rat and this isolated incidence in one generation only are considered to be of no toxicological significance.

Developmental studies were performed in rats by gavage at doses ranging from 5 to 120 mg/kg bw/d.

Maternal toxicity started at 10 mg/kg bw/d as suggested by the reduced body weight gain going to a reduced body weight when the dose increases. Reproduction parameters were not affected. A significantly increased post-implantation loss was reported at 120 mg/kg bw/d. At 60 mg/kg bw/d, one female had complete resorption. At 120 mg/kg bw/d, the number of live fetuses was reduced and mean fetal weight was significantly lower.

Fetotoxicity was evident at 80 mg/kg bw/d with a reduced mean fetal weight. At 40 mg/kg, bw/d there was also some evidence for fetotoxicity.

Major defects were reported at 120 mg/kg bw/d, two fetuses/litter exhibited a meningocele (neural tube closure defect) and another fetus had bilateral microphthalmia. At 80 mg/kg bw/d, one fetus/1 litter had a meningocele.

At 60 mg/kg bw/d 5 fetuses/5 litters were affected: defects of eyes (3 fetuses with other head defects); 1 fetus had a shortened jaw and cleft lip, 1 had meningocele, 1 fetus had unossified 2nd, 3rd and 4th arches of the cervical vertebrae, and one fetus displayed an abnormal zygomatic arch. None of which has been seen historically in controls in the laboratory. At 20 mg/kg, one fetus had four major defects, including a shortened lower jaw, which has not been seen in historical data. At 5 mg/kg bw/d, two fetuses in the 5 mg/kg group had abnormal zygomatic arch. Whilst this defect was not seen at 20 mg/kg, a single incidence was seen at 60 mg/kg.

The incidence of specific major defects and number of fetuses affected at 5 and 20 mg was small, not statistically significant, and did not form part of any dose-response relationship, although the possibility of a treatment-related effect cannot be completely dismissed.

Skeletal anomalies and variations were detected in the vertebral column and in sternum and the number affected litters were increased as compared to the control and sometimes higher at 40 mg/kg bw/d than at 120 mg/kg bw/d. These effects were assessed as sign of immaturity of fetuses. There was a dose-related effect for retardations. Retarded ossification of fetal skeletons, especially the incomplete ossification of skull bones could be connected with the mechanism of development of meningocele and influence of metam sodium cannot be excluded.

In rabbits, metam sodium was given by gavage at doses ranging from five up to 100 mg/kg bw/d.

Body weight gain of dams at 100 mg/kg bw/d was lower on most days of treatment. The influence on body weight gain partly corresponds with the diminished feed consumption. At 60 mg/kg bw/d, dams lost considerable weight after starting of dosing and food consumption was reduced. Similar but less marked changes were seen in the 20 mg/kg bw/d group.

At 100mg/kg bw/d, the number of dead implants was increased, especially due to the high number of early resorptions. Increased post implantation loss was seen at 100 and 60 mg/kg bw/d, and reduced number of live fetuses/pregnant female was seen at 30, 60 and 100 mg/kg bw/d. At 60 mg/kg bw/d, fetal weight was decreased. Percentage of male fetuses was reduced and litters containing five or less fetuses did not included males.

Two fetuses in two litters of 100 mg/kg bw/d showed a meningocele or spina bifida. These are rare anomalies in the strain of rabbits used in this study. There was a slight dose-related increase in sternebra asymmetry. The influence of metam sodium cannot be ruled out.

In each of the reported metam sodium studies, single cases of rare anomalies, reaching no statistically significant, were reported at maternal toxic doses and influence of metam sodium cannot be excluded. RMS proposes to classify metam sodium **Repr.Cat 3; R63**.

Similar defects affected head of rat and rabbits and were related to neural tube closure or eyes defects. Fetuses showed retarded ossification in head but also in limbs, thoracic vertebrae, sternum, and these effects were seen at doses with maternal toxicity.

Table B.6.6.3-1: Summary of reproductive toxicity and teratogenicity of Metam

Type of test Test species	Test substance purity	Results			References
		NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Symptoms	
2 generation rat study in drinking water at 0.01, 0.03 or 0.1 mg/ml	batch ref BAS/005/00N; 526 g/L or 43.148%/w; CTL n0 Y06930/008	NOAEL reproduction toxicity >0.1 mg/ml = 12 mg/kg bw/d. NOAEL fetotoxicity= 0.03 mg/ml = 4 mg/kg bw/d NOAEL parental toxicity= 0.03 mg/ml = 4mg/kg bw/d	- 0.1 mg/ml =12.3 mg/kg bw/d 0.1 mg/ml	- ↓ pup and litter weight ↓ body weight during premating, gestation and lactation	Milburn, 1993
Developmental rat study, gavage at 0, 10, 40, 120 mg/kg bw/d day 6-15 gestation	metam sodium batch n° ZH 130585; 42.2% or 517.3 g/L	NOAEL maternal toxicity= 10 mg/kg bw/d NOAEL developmental toxicity=10 mg/kg bw/d	40 mg/kg bw/d 40 mg/kg bw/d	↓body weight, body weight gain, food consumption ↑incidence of variations and retardations at maternal toxic doses; rare anomalies at top dose	Hellwig and Hildebrand, 1987
Developmental rat study, gavage 0, 5, 20 or 60 mg/kg bw/d days 7-16	metam sodium (ref n° BAS/005/00N, CTL n° Y06930/008; 43.15% w/w)	NOAEL parental toxicity= 5 mg/kg bw/d NOAEL developmental tox=5 mg/kg bw/d	20 mg/kg bw/d 20 mg/kg bw/d	- ↓body weight gain, food consumption, piloerection, salivation and urinary incontinence ↓ossification consistent with ↓ fetal weight Major defects were observed at 60 mg/kg bw/d Developmental toxicity: Cat 3, Xn, R 63	Tinston, 1993
Developmental rabbit study, gavage 0, 5, 20	metam sodium (ref n° BAS/005/00N,	NOAEL parental toxicity= 5	20 mg/kg bw/d	↓body weight gain, food consumption,	Hodge, 1993

Type of test Test species	Test substance purity	Results			References
		NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Symptoms	
or 60 mg/kg bw/d days 8- 20	CTL n° Y06930/008; 43.15% w/w)	mg/kg bw/d NOAEL developmental tox=20 mg/kg bw/d	60 mg/kg bw/d	piloerection, salivation and urinary incontinence ↓ fetal weight; altered sex ratio Major defects were observed at 60 mg/kg bw/d Developmental toxicity: Cat 3, Xn, R 63	
Developmental rabbit study, gavage 0, 10, 30 or 100 mg/kg bw/d days 6-18	metam sodium (ref n° 85/232, batch n° ZH130585; 42.2% w/w)	NOAEL parental = 10 mg/kg bw/d NOAEL developmental =10 mg/kg bw/d	30 mg/kg bw/d 30 mg/kg bw/d	↓ body weight gain ↓ number live fetuses; ↑ dead implants Major defects were observed at 100 mg/kg bw/d Developmental toxicity: Cat 3, Xn, R 63	Hellwig, 1987

In a 2 generation rat drinking water study, MITC was given at 2, 10, 50-ppm corresponding to 0.13-5.63 mg/kg bw/d. Variations in weight gain were observed in both sexes at top dose which occasionally attained statistical significance. Gestation and lactation weight were not altered. Mating performance and fertility were not adversely affected. The number of pups born/female was marginally lower at 10 and 50 ppm without reaching statistical significance. Physical and functional development of pups was comparable amongst all groups. Slight delays in the onset of eye opening and pinna unfolding were observed at top dose but were considered incidental to treatment, as there were no delays in the completion of each parameter. Functional development of top dose pups was comparable to controls on days 1, 17 and 21.

In a developmental rat study, MITC was given by gavage at 0, 3, 10 or 30 mg/kg bw/d. At the end of the treatment, several dams at top dose had sticky and/or moist fur in the area of the snout before, and at the same location reddish but mainly dry fur. Body weight and body weight gain were significantly reduced at top dose and marginally at intermediate dose. Water consumption was increased in individual dams at 10 and 30 mg/kg bw/d. Reproduction data were not significantly affected in the groups. The number of foetuses weighing < 75% of the mean fetal weight/litter was increased at top dose. Placenta weight was significantly lowered at top dose. Sex distribution was comparable in the different test groups.

One anomaly (anophthalmia) was detected in one foetus/one litter at 10 mg/kg bw/d. No retardations were seen in any group. There were no statistically significant differences between the treated groups and the controls with regard to anomalies, variations and/or retardations.

In rabbits, MITC was given by gavage at 1, 3 or 10 mg/kg bw/d. No test article related signs or symptoms were observed in any female of the different groups. At top dose, a slightly higher reduction of mean body weight was considered to be compound-related. Food consumption was reduced at top dose from day 6-11.

No effects were seen on the mean number of implantations, pups or embryonic deaths. Body weight of fetuses were not affected. Sex ratio was similar in all groups. Investigations of the crania and body cavity and skeletal of the fetuses did not show compound-related effects.

Table B.6.6.3-2: Summary of reproductive toxicity and teratogenicity of MITC

Type of test Test species	Test substance purity	Results			References
		NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Symptoms	
2-generation rat study, drinking water at nominal concentration of 2, 10 or 50 ppm	Batch n° 340178; purity?	NOAEL parental toxicity= 10 ppm =0.76-1.01 mg/kg bw/d) NOAEL reproduction toxicity > 50 ppm =3.6 mg/kg bw/d NOAEL offspring toxicity >50 ppm (3.6 mg/kg bw/d)	50 ppm (3.6 mg/kg bw/d) - -	↓ body weight gain -	Barker (1987), Barker (1986) and Jackson (1988)
Developmental rat study, gavage at 0, 3, 10 or 30 mg/kg bw/d day 6-15 gestation	Batch n° ZH 6205 MK; 96.9%	NOAEL maternal toxicity= 3 mg/kg bw/d NOAEL developmental toxicity=10 mg/kg bw/d	30 mg/kg bw/d 10 mg/kg bw/d	↓ body weight gain, ↑ water consumption ↓ fetal weight	Hellwig and Hildebrand, 1987
Developmental rabbit study, gavage 0, 1, 3 or 10 mg/kg bw/d days 6- 18	Batch n° ZNT 85/231-2; 98% w/w)	NOAEL parental toxicity= 3mg/kg bw/d NOAEL developmental tox=10 mg/kg bw/d	10 mg/kg bw/d -	↓ body weight and body weight gain no effects	Becker et al, 1986

B.6.7a Neurotoxicity studies of metam-sodium (Annex IIA 5.7)**B.6.7.1a Acute neurotoxicity studies (Annex IIA 5.7)**

- **An acute neurotoxicity study of Metam-Sodium in rats and the effect of Metam-Sodium on cholinesterase following acute oral exposure in the rat(Lamb, 1993)**

Findings:

Mortality: was observed at top dose: three males and two females died on day one; two males died on day 2 and the remaining female died on day 6. This effect was related to a primary systemic effect of metam

Clinical signs: were seen at top dose essentially occurring on the day following dosing.

Necropsy findings: in dead animals, clear fluid contents in the thoracic or abdominal cavity were observed for three rats. Three rats had reddened adrenal glands. One male had reddened mucosa in glandular portion of stomach and two males had a distended stomach. Pale lungs were noted in two males.

Body weight gain was reduced in the 750 and 1500 mg/kg bw group in male and female rats. Mean body weights were lower than control on day 7 for the 750 mg/kg males and on day 7, 14 and 15 for the 1500 mg/kg group males.

FOB: differences were observed between rats that received 750 and 1500 mg/kg and controls. In general, responses occurred approximately 45 minutes after treatment on day 0, and were transient in nature.

Based on the results of the functional observation battery and motor activity evaluations, all six of the functional domains (autonomic, activity, sensorimotor, physiological, neuromuscular and excitability) were affected by a single oral dose of metam at 750 and 1500 mg/kg bw. The activity domain (motor activity) was affected at 50 mg/kg bw.

Cholinesterase was not inhibited.

No signs indicative of neurotoxicity were observed at any dose level. The adverse effects observed at all dose levels were attributed to systemic toxicity and /or local gastrointestinal irritation of metam sodium.

No treatment related microscopic lesions were observed in any of the central or peripheral nervous system tissues in top dose animals.

Table B.6.7.1-1a: acute neurotoxicity study in rats with metam sodium.

Endpoints/dose	0		50		750		1500 mg/kg bw	
	M	F	M	F	M	F	M	F
Mortality							5	3
Clinical signs:								
Gait alteration						1	5	6
hypothermia							2	6
hypoactivity							2	3
ptosis							2	2
Pale + shallowed respiration								1
Body surface with red/yellow/orange material					10	7	10	13
Abnormal excreta						3	5	10
Body weight: day 7					↓10%*		↓13.5%*	
Bw gain: day 0-7					↓53%*	↓56%*	↓92%*	↓90%*
Bw changes day 0-15					↓17%*	↓28%*	↓33%*	↓25%*
FOB:								
Home cage observation: altered posture/palpebral closure					x	x	x	x
Handling observation: lachrimation, salivation, altered palpebral closure, change in fur appearance and respiration rate					x	x	x	x
Open field observation:								
Increased time for first step						x	x	x
Gait and arousal alterations and					x	x	x	x

Endpoints/dose	0		50		750		1500 mg/kg bw/d	
	M	F	M	F	M	F	M	F
decreased rearing activity								
Sensory observations:								
Absent olfactory/pupil response					x	x	x	x
Absent tail pinch response					x	x	x	x
Slower reaction to tail pinch stimulus						x		x
Reduced startle response					x	x	x	x
Neuromuscular observations:								
Reduced hindlimb extensor strength					x	x	x	x
Physiological observations:								
Decreased body temperature					x	x	x	x
Decreased body weight					x day7/14		x day7/14	x day 7
Locomotor activity:								
Mean ambulatory activity reduction day 0			x	x	x	x	x	x
Day 7 / 14								x
Total motor activity counts day 0			x	x	x	x	x	x
Day 7 / 14								x
Cholinesterase activity Day 0								
Plasma U/L	743	1243					833	1424
RBCs U/L	6938	6826					6181	6044
Hippocampus U/g	29730	30072					34315	36329
Olfactory region U/g	25745	27531					28538	25706
Midbrain U/g	13363	14135					13454	14382
Brain stem U/g	18849	19653					18111	20022
Cerebellum U/g	12407	12880					12224	13169
Cerebral cortex U/g	3417	3439					3467	3665
Cholinesterase activity Day 1								
Plasma U/L	753	1414					710	1079
RBCs U/L	6850	6789					6049	5840
Hippocampus U/g	32011	30905					29473	38658
Olfactory region U/g	24250	27349					29214	27127
Midbrain U/g	13222	14398					13110	13541
Brain stem U/g	19012	20470					17890	19820
Cerebellum U/g	12180	13956					11689	13565
Cerebral cortex U/g	3404	3425					3447	3816

*: statistically significantly different from control; x: effect is observed.

Conclusion:

The company concluded that clear signs of acute systemic toxicity were observed at the different dose levels tested in this study.

NOAEL systemic toxicity < 50 mg/kg bw/d.

No neurotoxicity was seen in this study. NOAEL acute neurotoxicity > 1500 mg/kg bw/d.

RMS agrees that this study is conducted at very high dose levels and it is difficult to differentiate between direct and indirect effects on the nervous system. Changes in motor activity reflect an effect on the nervous system only in the absence of general toxicity. In any case, if metam should be considered as a neurotoxic substance, the effects reported in this study are observed at doses that are above the NOAEL for other toxic effects. Therefore, RMS concludes that the effects on the nervous system seen in this study are secondary to systemic toxicity.

GLP status: yes (no attest of competent authority)

Experimental protocol: not fully in agreement with OECD test guideline 424 (1997).

Deviation from official protocol: doses were too high. Muscle histological section was not performed. Sections including hippocampus and retina were not included.

Material and methods: 16 Sprague-Dawley rats/sex/dose received by gavage a single oral dose of metam (batch n° 43.15% w/w) at 50, 750 or 1500 mg/kg bw. A control group received water only. FOB and motor activity evaluations were performed on day 7 and 14. neuropathology was performed in five animals/sex in control and top dose rats. A separate group of 10 rats/sex received 1500 mg/kg metam and another group of 10 served as control. 45 minutes post dosing, blood was collected for plasma and RBC cholinesterase activity determination in five rats/sex at top dose and in control rats. The remaining five rats were sacrificed 24 h later. Brain cholinesterase was measured in olfactory, cerebral cortex, cerebellum, brain stem, hippocampus and mid brain at 24 h after dosing. Dosing method is not reported. The study is accepted.

B.6.7.2a Subchronic neurotoxicity studies (Annex IIA 5.7)

- Metam Sodium: Subchronic Neurotoxicity Study in Rats (Allen, 1994)

Findings:

Body weight was reduced in males at 0.2 mg/ml and in females of all treated groups. This reduction in body weight reflects probably the reduction in food consumption rather than toxicity.

Food consumption reductions were seen at top dose throughout the study and in females at 0.06mg/ml after week 1. Food consumption was reduced as a compensatory measure to reduced water consumption.

Food utilization was slightly reduced for males at 0.2 mg/ml during weeks 1-4 and overall and may reflect a minor toxic response to metam sodium

Water consumption in both male and female rats treated with metam sodium showed dose-dependent reduction throughout the study, reflecting the poor palatability of the test drinking water solutions.

Functional observation battery: a reduction in landing foot splay was seen in males at top dose in week 9 only. In the absence of any effect at other timepoints, particularly week 14, this difference is considered spurious and incidental to treatment. There were no effects on all the other measurements.

Rats revealed no evidence of peripheral and nervous system alterations attributable to treatment with metam sodium.

Table B.6.7.2-1a: 13-week neurotoxicity study in rats with metam sodium.

Endpoints/dose	0		0.02		0.06		0.2 mg/ml	
	M	F	M	F	M	F	M	F
Mean dose received mg/kg bw/d	0		2	3.3	6	8.4	14.7	17.8
Mortality							5	3
Body weight end of study:				↓6%*		↓8%*	↓9-10%*	↓9-10%*
Food consumption				↓6%* wk 5-6,13		↓6%* wk 1	↓6%*	↓6%*
Food utilization						↓9%* wk 1-13	↓6%*	
Water consumption wk 13			↓12%*	↓28%*	↓12%*	↓37%*	↓47%*	↓65%*
FOB:								
Clinical conditions			There were no treatment related observations					
Landing foot splay measurements							↓* wk 9	
Time to tail flick			There were no treatment related observations					
Grip strength measurements			There were no treatment related observations					
Motor activity measurements			There were no treatment related observations					
Brain measurements			There were no treatment related observations					
Macro/microscopic findings			There were no treatment related observations					

*: statistically significantly different from control.

Conclusion:

Metam sodium is not neurotoxic under these experimental conditions. The NOAEL for neurotoxicity = 0.2 mg/ml metam sodium corresponding to 14.7 mg/kg bw/d.

GLP status: yes (no attest of competent authority)

Experimental protocol: not fully in agreement with OECD test guideline 424 (1997).

Deviation from official protocol: animal observation was limited to 1/day. FOBS were not performed before 5-week exposure. Histopathology was performed only for control and top doses.

Material and methods: 12 Alpk: APfSD strain rats/sex/dose received in their drinking water metam sodium (batch ref n° BAS/005/00n 90-2; 43.148% w/w) at 0, 0.02, 0.06 or 0.2 mg/ml for 13 weeks. Samples of drinking water were taken at intervals throughout the study and analyzed to establish the achieved concentration. The achieved dose levels were within 20% of the target level and the overall mean achieved concentrations were within 10% of the target concentrations. Reduction in stability was less than 15%.

The study is accepted.

B.6.7.3a Summary of neurotoxicity studies (Annex IIA 5.7)

An acute neurotoxicity study was performed with metam sodium administered to rats at 50, 750 or 1500 mg/kg bw by gavage. In this study, a NOAEL systemic toxicity < 50 mg/kg bw/d was proposed and no neurotoxicity was seen in this study. NOAEL acute neurotoxicity > 1500 mg/kg bw/d. This study was conducted at very high dose levels and it is difficult to differentiate between direct and indirect effects on the nervous system. In any case, if metam should be considered as a neurotoxic substance, the effects reported in this study are observed at doses that are above the NOAEL for other toxic effects. Therefore, RMS concludes that the effects on the nervous system seen in this study are secondary to systemic toxicity.

In a repeat dose study, where metam sodium was administered in drinking water at doses going to 0.2 mg/ml (15-18 mg/kg bw/d), there were no signs of neurotoxicity.

B.6.8 Further toxicological studies (Annex IIA 5.8)

From the open literature:

- Role of decomposition products in sodium methyldithiocarbamate- induced immunotoxicity (Keil et al., 1996)

Findings:

According to the authors, the most prominent effects of metam include a decrease in thymus weight and percentage of CD4⁺CD8⁺ thymocytes, an increase in spleen weight, in the percentage of neutrophils in the blood, and a decrease in the percentage of lymphocytes in the blood, and in natural killer (NK) cell activity in the spleen.

Equimolar dosages of methylamine and carbon disulfide caused minimal immunological changes, and these changes were not characteristic of those noted for metam sodium. In contrast, MITC significantly decreased thymus weight and cellularity and changed peripheral white blood cell populations in a manner similar to that noted for an equimolar dosage of metam sodium. However, MITC did not significantly affect NK cell activity or increase spleen weight. MITC, methylamine, or carbon disulfide did not produce the remaining change. Thus, it is likely that the parent compound or a synergistic action of the parent compound with one or more of the decomposition products is responsible for these remaining changes (increased spleen weight and decreased splenic NK cell activity). In rats as well as mice, SMD-induced thymus atrophy occurs and the dosage required to decrease thymus weight by 50% is lower for rats than for mice.

Conclusion:

Metam sodium potential as a human immunotoxicant needs to be further investigated as well as the role of MITC in the immunotoxic effects of SMD in different species.

According to RMS: While metam sodium at 300 mg/kg and methylamine up to 978 mg/kg bw were without effects on blood leukocytes number, MITC at 30 mg/kg bw, induced an increase, which was not dose-related. Neutrophil count was not affected while lymphocyte percentage was slightly increased after metam or MITC, but not with a dose related effect. Absolute thymus weight was reduced after metam sodium and MITC. CS₂ increased thymus weight (relative organ weights are not reported). Methylamine was without effect.

In the studies provided by the notifier, metam sodium and MITC had toxic effects at much lower doses and effects on lymphocytes were sometimes reported. Thymus involution was observed in a 90-day dog study with MITC. RMS considers that the effects reported in this published study are probably due to a high systemic toxicity and not a direct effect of metam or MITC.

Material and methods: Five female B6C3F1 mice and five female Fisher rats were used and received a daily dose of one of each compounds for 5 days by gavage unless otherwise specified. A control group was included.

Metam sodium (Sigma Chemical, purity?) was given at 300 mg/kg.

MITC (Sigma Chemical, purity?) was prepared in Hanks balanced salt medium and administered at a dose range of 15, 30, 45 mg/kg

Methylamine (Sigma Chemical, purity?) was administered in a Hanks balanced salt solution by ip route at 122, 489 and 978 mg/kg/d.

CS₂ (Sigma Chemical, purity?) was prepared in an aqueous solution of 0.5% CMC and 0.1% Tween 80 and administered at 300 mg/kg, 551 and 1102 mg/kg.

Blood was collected on day 6 following the 5 d of dosing. Thymocytes were analyzed by flow cytometry after preparation of a single cell suspension from each thymus. NK cell activity was assessed after 5 day by measuring the lyses of ⁵¹Cr-labeled YAC-1 tumor cells by splenocytes from spleen suspensions.

This study is not relevant for the dossier.

B.6.8.1 Toxicity studies on metabolites and impurities (Annex IIA 5.8.1)

B.6.8.1.1 toxicity studies on metabolites of metam sodium and MITC (Annex II A 5.8.1)

When metam sodium decomposes, a complex mixture of irritant compounds is formed: the primary pesticidal agent MITC that could further decompose in methyl isocyanate (MIC), carbon disulfide, hydrogen sulfide and methylamine. The specific mix of products depends upon soil pH and other environmental conditions.

The toxicological effects of the mixture cannot be characterized, at least limited information is available for each individual compounds.

Thus, while metam sodium is itself non-volatile, its application results in significant production and emission of air contaminants. The half-life of MITC in air was reported as 29 to 39 hours in natural sunlight. MITC in air degrades by photolytic decomposition, in part to methyl isocyanate (MIC). MIC may be photochemically stable.

Therefore, following agricultural metam-sodium applications, inhalation exposure is essentially related to MITC, but might also include other degradation products such as CS₂, H₂S and MIC. There is uncertainty about how these breakdown products interact to produce the overall potential toxicity deriving from the use of metam-sodium, but MITC, MIC and H₂S have all been associated with ocular and respiratory irritation. While there are no data to address mixed exposure, additive or synergistic effects of MITC, MIC and H₂S in respiratory and ocular tissues are plausible.

A quite complete dossier related to **MITC** is included in the DAR. For MIC, the company did not provide studies. Therefore, it was necessary to do a search in the open literature. Some of the published studies are summarized here. ECB (Ispra) reported a rationale for classification of methylisocyanate. A summary of the rationale for classification is also proposed below.

However, the company to evaluate the potential exposure of operator to MIC, CS₂ and methylamine, performed an air monitoring study after metam sodium application with a tractor mounted Rumpstad shear injector (see point B.6.15.2.2 study of Links, 2006). From this study, it was concluded that the potential exposure of the operator to MIC and the stationary measurements to MIC inside the tractor were both below the limit of detection.

MIC, CS₂ and methylamine were not detected around the treated field. At detection limit, the upper bound estimate for results of MIC for all field samples would be 4-µg/m³. For CS₂ this would be 400-µg/m³ and for methylamine, this would be 27-µg/m³. Air concentrations of MITC ranged from the limit of detection (0.042µg/m³) to a maximum concentration of 36-µg/m³ with a GM of 0.9 µg/m³ (n=96). The 95th percentile was 13.2 µg/m³.

From the open literature:

Methylisocyanate (MIC):

MIC, like reactive isocyanides in general, can react with biological molecules containing amino, alcohol, or sulfhydryl groups, as well as with water. While hydrolysis in an aqueous environment such as the lung, is theoretically possible, measurements show that alkylisocyanates are relatively resistant (compared to arylisocyanates) to such hydrolysis (determination of No cancer chronic reference exposure levels batch 2B, December 2001).

1 ppm MIC = 2.33 mg/m³

1) Human data from the open literature:

MIC is highly toxic. Accidental release of 30 to 35 tons of MIC from a pesticide factory in Bhopal, India, caused thousands of deaths by acute respiratory failure. Survivors suffered skin and eye injuries, shortness of breath, chest pains, cough, throat irritation, choking and hemoptysis (expectoration of blood). Objective signs of the corrosive effects of MIC on the respiratory tract were interstitial and alveolar edema and destructive lung lesions with cavitations, alveolar wall thickening and interstitial fibrosis. Pulmonary function tests indicated lung volume, airflow and pulmonary vascular impairments. Bronchiolitis obliterans was a long-term result of the acute lung injury. MIC exposure concentrations in the Bhopal accident were estimated to be between 13 and 100 ppm. Many of the acute and chronic signs observed in humans have been reproduced in experimental laboratory experiments (mice and rats).

- In three controlled exposures of human volunteers to MIC, eye irritation and lachrimation were observed after exposures ranging from 0.5 ppm to 5-ppm for 10 seconds to 50 minutes.

- MIC exposure has severe consequences for fetal and neonatal survival. In Bhopal, fetal loss rose from an estimated normal incidence rate for that area of 6-10%, to 43% in the exposed population. Mortality among infants exposed *in utero* increased over four-fold, from 2.6-3% in the 30 days after birth during the 2 years preceding the accident, to 14% after the disaster (Froines, 2002).

2) Animal data from the open literature:

Single dose inhalation toxicity, 3, 10 ppm:

- **Medical Toxicology Branch, Department of Pesticide Regulation California Environmental Protection Agency, RISK CHARACTERIZATION DOCUMENT Methyl Isothiocyanate (MITC) Following the Agricultural Use of Metam Sodium(July 25, 2003)**

Findings and conclusion: NTP performed a kind of long-term study with MIC but at a single oral dose. Groups of 50 or 100 six to eight-week-old F344 rats and B6C3F1 mice were exposed once to MIC by inhalation at 0, 3, or 10 ppm for 2 hours. After 2 years, animals were sacrificed and tissues and organs were examined microscopically. No differences in survival rates or body weight gains were found in MIC exposed animals versus controls. Male and female rats exposed to 10 ppm MIC had 42% and 36% incidence, respectively, of intraluminal fibrosis of lung secondary bronchi; no evidence of this lesion was seen in controls or animals exposed to lower concentrations. For male and female mice and female rats, no neoplastic lesions were significantly associated with MIC exposure. Male rats exposed to MIC had marginally increased rates of pheochromocytomas of the adrenal medulla, and adenoma of pancreatic acinar cell.

- **Pathologic changes following acute methyl isocyanate inhalation and recovery in B6C3F1 mice (Boorman et al, 1987)**

Findings and conclusion: Sixteen of 80 (20%) male mice in the 30-ppm group died following exposure with seven deaths occurring in the first 24 hr. Two female mice died, one each in the 30- and 10-ppm concentration groups. Treatment-related changes were restricted to the respiratory system. At 30 ppm, there was extensive necrosis and erosion of the respiratory and olfactory epithelia in the nasal cavity. Severe necrosis and epithelial erosion were also found in the trachea and main bronchi. Epithelialization occurred rapidly and most of the denuded turbinates were covered by flattened epithelium by Day 3. On the turbinates, recovery was essentially complete with the exception of small areas in the olfactory epithelia where lesions were present in the male mice through Day 91. In the trachea and major bronchi, fibrin and cellular debris were present in the airways, which in some cases had organized and formed intraluminal fibrotic projections. These projections became covered by respiratory epithelium. The intraluminal projections and bronchial fibrosis persisted to Day 91. In the males, where the fibrosis was more severe, chronic alveolitis and atelectasis were found. In mice exposed to 3 or 10 ppm, there appeared to be complete recovery. These inhalation studies indicate that methyl isocyanate exposure of mice at or near lethal concentrations causes reversible lesions in the nose and persistent intraluminal and mural fibrosis of the major bronchi.

Material and methods: Male and female mice were exposed by inhalation to 0, 3, 10, and 30 ppm methyl isocyanate for 2 hr followed by a 91-day recovery period. Five mice/sex/group were examined at 2 hr and at 1, 3, 7, 14, 28, 49, and 91 days following exposure.

4-day inhalation, 1 or 3 ppm:

- **Four-day repeated inhalation and recovery study of methyl isocyanate in F344 rats and B6C3F1 mice (Mitsumori et al, 1987)**

Findings: 49 of 56 male rats, 51 of 56 female rats, and 1 of 56 male mice in the 3-ppm group died by 28 days; early death animals were also examined histological.

Reduced *weight gain* was observed among the female and male rats in the high dose group, prior to death, although the absolute weights were not different from the unexposed rats one day before the end of exposure. Among the mice, a slowed weight gain was observed at 3- and 6-days post exposure (male) and 1 day post exposure (female) at the high dose, but normal weight gain returned by 1 week following cessation of exposure. Histopathological findings: Exposure-related changes occurred in rats and mice of both sexes in the 3-ppm group only.

Lesions of the nasal cavity in rats and mice were characterized by regeneration of the olfactory and respiratory epithelia secondary to epithelial erosion. By Day 28, the olfactory and respiratory epithelia in mice appeared normal, while in rats incomplete regeneration of the olfactory epithelium was still present.

Regeneration of the respiratory epithelium in the trachea of rats occurred in the 3-ppm group and the epithelium appeared to return to normal by Day 28.

Lung lesions in rats consisted of mural and/or intraluminal fibrosis secondary to extensive erosion of the respiratory epithelium in the major bronchi to the terminal bronchioles. Acute inflammation of the small airways, occasional hyaline membranes of alveolar walls, and pulmonary atelectasis were also seen. Alveolar fibrosis was observed in rats found dead from Day 14 on and in male rats killed on Day 28.

Atrophy of the thymus and spleen, atrial thrombosis of the heart, and hepatocellular necrosis were frequently seen in rats dying following MIC exposure. The lung lesions in mice were qualitatively similar to those in rats, but were restricted to the major bronchi. Minimal intraluminal or mural fibrosis was still present in mice on Day 91. In a separate study, a single 6-hr exposure of five male rats to 3 ppm MIC was followed by a recovery period of 7 days. The lesions of the respiratory system were essentially the same as those in the 3-ppm group killed on Day 7 after the 4-day repeated exposure of MIC, but the alveolar lesions were more severe. These data suggest that the rat respiratory tract is more sensitive to MIC than of the mouse. A LOAEL of 3 ppm is indicated, based on post-exposure decreased weight gain and respiratory tract changes in rats. NOAEL = 1 ppm.

Material and methods: F344 rats and B6C3F1 mice of both sexes were exposed by inhalation to zero, 1, and 3 ppm methyl isocyanate (MIC) for 4 consecutive days (6 hr/day) followed by a recovery period of 91 days. Five mice and rats/sex/group except the 3 ppm group (5 rats/ sex on Day 7 and 2 males on Day 28) were killed on Days 7, 28, 49, and 91 after the exposure and examined histopathologically.

- 6 days inhalation, 0.212, 0.265, and 0.349 ppm:

Acute and subacute toxicity study of inhaled methyl isocyanate in Charles Foster rats (Sethi, 1989)

Findings:

Charles Foster male and female rats were exposed to MIC at 0.212, 0.265, and 0.349 ppm doses for 30 min daily, for 6 days, by inhalation. During exposure, animals were observed to have congestion in eyes, lachrymation, nasal secretion and dyspnea, progressively increasing ataxia, and immobility. Uncoordinated movements were also observed, indicating effects on the nervous system. Upon microscopic examination of the viscera, pathological findings confined to bronchial tree, lung parenchyma, liver, and kidneys were observed. At the lowest dose (0.21 ppm), the weight gain was 111 g after 91 days post-exposure, compared to a weight gain of 218 g during the same interval among the non-exposed rats. According to the narrative, inflammatory lesions of bronchopulmonary tissue were present; their extent increased with dose. A dose-response increase in markers of lung infection was present and suggests that the MIC exposed rats were more prone to infectious agents than were the unexposed animals. Non-specific lesions in liver and kidneys were also observed and appeared to be dose dependent, but the authors suggested that these effects could be a result of the lung infections.

Conclusion: NOAEL < 0.212 ppm

Material and methods: Charles Foster male and female rats were exposed to MIC at 0.212, 0.265, and 0.349 ppm doses for 30 min daily, for 6 days, by inhalation. Clinical signs, mortality, body and organ weights, and changes in hematology and clinical pathology were routinely monitored to determine the principal organ sites damaged on exposure to the gas.

2x4 days inhalation exposure, 0.15, 0.6 or 3.1 ppm:

- Methyl isocyanate subchronic vapor inhalation studies with Fischer 344 rats (Dodd and Fowler (1986))

Findings:

No deaths occurred at any MIC concentration during the exposure. Lesser weight gain occurred for rats in the 3.1-ppm groups, whereas weight among the rats in the 0.15 and 0.6 ppm MIC groups was indistinguishable from the air-exposed control animals. On exposure days three and eight, mean food consumption values in the high dose group were below those for the non-exposed group. At the time of termination, male rats exposed to 3.1 ppm MIC exhibited an increase in hemoglobin concentration and a 26 % decrease ($p < 0.001$) in oxygen saturation, compared to the unexposed rats ($p < 0.001$). Such changes were not observed for the female rats exposed to 3.1 ppm or for rats of either sex exposed to 0.15 or 0.6 ppm MIC. Absolute lung weights increased ($p < 0.001$) in both sexes after exposure to 3.1 ppm, compared to the control rats. Decreases in liver, kidney and testes absolute weights were observed in this exposure group, but the authors interpreted these data as a reflection of the body weight losses. No weight changes were observed in rats exposed to 0.15 or 0.60 ppm MIC. Gross and microscopic lesions were observed in rats (female and male) exposed to 3.1 ppm, but not in rats exposed to 0, 0.15, or 0.6 ppm MIC. The microscopic lesions occurred in the respiratory tract and consisted of inflammation, epithelial necrosis, squamous metaplasia, and epithelial hyperplasia. These lesions extended into the bronchioles.

The early lesions associated with inflammation, epithelial necrosis, squamous metaplasia, and epithelial hyperplasia extending to the bronchioles either decreased in severity or receded toward the upper respiratory tract by 85-days post-exposure. In males, the intraluminal and submucosal fibroplasias changed in appearance during this interval, due in part to the maturation of fibrous tissue. Mucous plugs were also seen in the terminal bronchioles and alveoli in some rats. The importance of this observation is the progressive character of MIC induced lung disease. Such progression may be difficult to follow at lower doses, if the times involved are of insufficient duration. Post-exposure evaluation showed that, at a higher exposure level (3.1 ppm), progressive changes, including death, occurred. Post-exposure observations, however, were not reported at the 0.15 and 0.6 ppm MIC levels.

These data suggest a NOAEL of 0.6 ppm MIC (0.335 mg/kg bw).

Table B.6.8.1.1-1: findings in rats after exposure to MIC:

Endpoints/dose	0		0.15		0.6		3.1 ppm	
	M	F	M	F	M	F	M	F
N° examined rats	10	10	10	10	10	10	10	10
Body weight loss							↓	↓
Food consumption							↓	↓
Blood analysis:								
Hemoglobin concentration							↓38%*	
Oxygen saturation							↓26%*	
Nasal passage								
Rhinitis	1	1	0	2	1	2	9*	10*
Epithelial degeneration/necrosis	0	0	0	0	0	0	0	4
Squamous metaplasia	0	0	0	0	0	0	10*	10*
Trachea								
Tracheitis	0	0	0	0	0	0	9*	6*
Ulceration	0	0	0	0	0	0	1	0
Squamous metaplasia	0	0	0	0	0	0	10*	9*
Regenerative hyperplasia	0	0	0	0	0	0	1	1
Lung/bronchioles								
bronchiolitis	0	0	0	0	0	0	10*	9*
pneumonitis	0	0	0	0	0	0	5*	7*
Squamous metaplasia	0	0	0	0	0	0	4	9*
Intraluminal /submucosal fibroplasia	0	0	0	0	0	0	4	0
Bronchial lymph nodes								
Reactive hyperplasia	0	0	0	0	0	0	5*/8	5*/7

Table B.6.8.1.1-2: post exposure examination of rats exposed to 3.1 ppm MIC on days 1, 15, 43 and 85.

Endpoints/sacrifice time (days)	1		15		43		85	
	M	F	M	F	M	F	M	F
Mortality %	0	0	63	6	52	3	0	0
Nasal passage								
Rhinitis	7/8	8/8		1/10	4/6	0/10	2/3	1/10
Ulceration/erosion	0/8	0/8		0/10	0/6	0/10	0/3	0/10
Squamous metaplasia	8/8	8/8		7/10	6/6	4/10	3/3	1/10
Lung/bronchioles								
bronchiolitis	6/8	4/8		0/10	4/6	0/10	1/3	0/10
pneumonitis	4/8	2/8		4/10	5/6	0/10	2/3	0/10
Regenerative hyperplasia	2/8	7/8		10/10	5/6	6/10	3/3	1/10
Squamous metaplasia	5/8	7/8			2/6	0/10	0/3	0/10
fibroplasia	6/8	0/8		0/10	3/9	0/10	2/3	0/10

Conclusion:

NOAEL = 0.6 ppm (0.335 mg/kg bw/d) could be considered the most relevant for deriving a chronic reference exposure level based on the decreased weight gain and lung pathology immediately after cessation of exposure (LOAEL 3.1 ppm = 1.73 mg/kg bw/d).

Material and methods: Groups of Fischer 344 rats were exposed to 3.1, 0.6, 0.15, or 0.0 (control) ppm of methyl isocyanate (MIC) vapor 6 hr/day for two 4-day sessions separated by a 2-day rest.

The rats were killed the morning following the last exposure day.

In a second study, rats were exposed to 3.0 ppm MIC, 6 hr/day, for either one or two 4-day sessions and sacrificed on post-exposure Days 1, 15, 43, and 85.

Genotoxicity:

- **Inhalation toxicity of methylisocyanate: assessment of germ cell mutagenicity and reproductive effects in rats (Agarwal DK, Bose M. Indian J Exp Biol. 1992 Jun;30(6):504-8)**

Findings and conclusions: Sequential mating of treated males with normal females on days 1-7, 8-14 and 15-21 post-exposure did not indicate any induction of dominant lethal mutation (increased frequency of preimplantation losses and early fetal deaths) by MIC but it was significantly induced by EMS as compared to respective controls. Males, necropsied after 21 days of exposure, showed no effect of MIC on epididymal sperm density and morphology. EMS also had no effect on sperm density but it significantly induced morphological abnormalities in sperm as compared to untreated controls. There was an acute and transitional reduction in reproductive performance (10-21%) of MIC-exposed males during day's 1-14 post-exposure followed by recovery to the normal level during day's 15-21 post-exposure. The progeny of MIC-exposed males was also normal in terms of litter size, litter weight, neonatal survival and body weight gain in litters up to 10 days post-partum.

In conclusion, the observed failure of MIC to cause germ cell mutagenicity is related to its poor biodistribution to the target site(s) and a transient reduction in the reproductive performance of MIC-exposed males is a result of general stress and disconsolate copulation.

Material and methods: Adult male Wistar rats were exposed to methylisocyanate (MIC, 3.2 mg/l, single inhalation exposure for 8 min under static condition) or ethyl methanesulphonate (EMS, 150 mg/kg, single ip dose) for the assessment of germ cell mutagenicity and reproductive effects.

- **Results of the *in vitro* and *in vivo* genetic toxicity of MIC (Shelby et al, 1987)**

Findings:

Methyl isocyanate (MIC) was tested for genetic toxicity in a variety of *in vitro* and *in vivo* assays.

Negative results were obtained in the *Salmonella*/mammalian microsome assay using five bacterial strains in a preincubation protocol.

The *Drosophila* sex-linked recessive lethal test also gave negative results in studies that involved three routes of administration: inhalation, feeding, and injection.

Positive results were obtained for three endpoints in cultured mammalian cells. Reproducible, dose-related increases in trifluorothymidine-resistant clones were induced in L5178Y mouse lymphoma cells, and the frequencies of both SCE and chromosomal aberrations increased in Chinese hamster ovary cells. These effects were independent of exogenous metabolism.

In mice exposed to methyl isocyanate by inhalation, cytogenetic analyses were carried out on bone marrow, blood, and lung cells. In a single, 2-hr exposure to concentrations of 0, 3, 10, and 30 ppm MIC produced no evidence of chromosomal effects in the bone marrow, although significant cell cycle delay was observed. In four experiments involving exposures on four consecutive days to zero, 1, 3, or 6 ppm, delays in bone marrow cell cycle were again observed.

Increases in SCE and chromosomal aberrations were observed in bone marrow cells, and a dose-related increase in SCE occurred in lung cells but not in peripheral blood lymphocytes. A significant increase in micronucleated polychromatic erythrocytes in the peripheral blood was observed in male mice in one experiment.

From these results, the authors concluded that methyl isocyanate has the capacity to affect chromosome structure but not to induce gene mutations. Furthermore, *in vitro* tests show that the induction of chromosomal effects is not dependent on an exogenous source of metabolism. Based on these results and on what is known

about the binding of carbamoylating agents to cellular macromolecules, methyl isocyanate may exert its genetic toxicity by binding to nuclear proteins rather than by binding to DNA.

ECB considered that the effect observed was a cytotoxic effect and not a genotoxic effect and did not propose a classification for genotoxicity.

Reproduction toxicity and developmental toxicity:

- **Epidemiological and experimental studies on the effects of methyl isocyanate on the course of pregnancy in mice (Varma, 1987)**

Findings:

In Female mice exposed for 3 hours on gestation day 8 to 15 ppm MIC, 4 retained pregnancy and 12 did not retain pregnancy. At 15, 9, 6, or 2-ppm concentrations, MIC caused a decrease in fetal and placental weight. Maternal body weight was not significantly affected. A meningocele was observed in one fetus at 9 ppm. Reduction in lengths of the mandible and bones of extremities was reported at 6 ppm onwards. After ip, administration of 80 mg/kg lethality was observed in all mice in less than 16 hr.

Table B.6.8.1.1-3: effects of MIC in mice.

Treatment /endpoint	none	air	2ppm	6ppm	9ppm	15ppm
N° animals	10	24	11	12	12	18
Maternal mortality	0	0	0	0	2	2
Surviving losing all embryos%	0	0	9	8	80	75
Fetal body weight g	1.5	1.5	1.4*	1.4*	1.1*	1.3*
Placental weight (g)	97	95	88*	85*	80*	84*

*Statistically significantly different from control

Conclusion:

After a single exposure of pregnant rats to MIC for 3 hr, there was a concentration dependent increase in embryo loss, starting from the lowest dose onwards. Placental weight was decreased in parallel. LOAEL_{feto/embryotoxicity} = 2 ppm. It is difficult to conclude for maternal toxicity as only body weight was measured and was not affected.

A meningocele was observed in one fetus at 9 ppm (5.7 mg/kg bw). Reduction in lengths of the mandible and bones of extremities was reported at 6 ppm onwards.

Material and methods: pregnant Swiss-Webster mice were exposed to MIC vapors once for 3 hr on day 8 of gestation and were sacrificed on day 18 of gestation. In a limited number of experiments, the effect of ip injection of liquid MIC into 8-day pregnant mice and 13-day pregnant rat was also studied.

- **Methylisocyanate: reproductive and developmental toxicology studies in Swiss mice (Schwetz et al, 1987).**

Findings and conclusions:

Among female mice exposed to one (0.63 mg/kg bw), or 3 ppm (=1.89 mg/kg bw) MIC during gestation days 14 – 17 for 6 hr/day, an increased incidence of fetal deaths was observed at 1 ppm. At 3 ppm, the average number of pups/litter decreased relative to the air-exposed controls. The dams were unaffected in terms of survival, body weight, or length of gestation.

Exposure of males alone (6 hr/day, 4 days) did not affect the number of pregnancies or the live litter sizes, suggesting that the fetotoxic effect may be specific to the female reproductive tract rather than a general attribute of systemic toxicity.

Exposure of male and female mice to 0, 1, or 3 ppm MIC did not result in altered body weights, fertility, or litter size. The results suggest that exposures to MIC at doses that are not toxic to adult male or female (pre-gestational) mice or rats do not result in adverse reproductive outcomes.

Material and methods: CD Swiss mice were exposed on GD 14-17, 6 hour/day to 0, 1 or 3 ppm MIC (UCC, 99%) and were allowed to deliver their offspring. Male mice were mated with untreated females for 8 weeks following the last of four consecutive days of exposure, 6 hr/day to 0, 1 or 3 ppm MIC.

3) ECB rational for classification of methyl isocyanate:

Some of the studies used by the ECB are reported above, under point 2.

Acute toxicity

Oral and dermal toxicity:

A classification with **T, R25** is proposed for oral toxicity.

No information has been found for dermal toxicity, so in the absence of other information, **T, R24** is maintained.

Inhalation toxicity:

4 hour LC50 rat, 0.026 mg/L (=7 mg/kg bw)

4 hour LC50 guinea pig, <0.012 mg/L (3.24 mg/kg bw)

6 hour LC50 rat, 0.014 mg/L (3.78 mg/kg bw)

6 hour LC50 mouse, 0.028 mg/L (7.56 mg/kg bw)

6 hour LC50 guinea pig, 0.013 mg/L (3.5 mg/kg bw)

MIC should be classified as **T⁺, R26**

Irritation:

Skin: **Xi, R38** is proposed based on a combined skin irritation and dermal sensitization test in guinea pigs.

Eye: it is proposed that the evidence from humans, especially, justifies classification with **R41**.

Respiratory tract: **R37**.

Sensitization: MIC is very reactive with tissue macromolecules and would be expected to be allergenic. **R42** is proposed, as the substance is an isocyanate.

R43 is recommended.

Genotoxicity:

ECB considered that MIC did not induce a genotoxic effect and did not propose a classification for genotoxicity.

Although the carcinogenic potential of MIC has not been fully evaluated, no classification is indicated based on the available data.

Conclusion:

- Human data suggest from the Bhopal event that exposure to air concentration of 13 to 100 ppm is extremely toxic.

- ECB proposed the following classification of MIC:

T⁺; R26

T; R24/R25

Xn; R42/43

Xi; R37/38/41

MIC has not shown an adverse effect on fertility where parental toxicity is absent. MIC is very toxic by inhalation, and even extremely low dose cause maternal toxicity and it is thus very difficult to determine if impaired foetal development can occur in the absence of maternal toxicity. The developmental studies in humans and animals do not give a definitive answer, but there is a strong suspicion that foetotoxicity is a non-specific consequence of maternal toxicity. Therefore, it is proposed not to classify MIC as reproductive hazard.

- Animal data reported in the open literature:

After a single exposure to MIC at 10 ppm for 2 hours, intraluminal lung fibrosis was observed 2 years later.

Exposure at 30 ppm for 2 hour was followed by extensive necrosis and erosion of respiratory and olfactory epithelium in the nasal cavity of mice.

Four consecutive 6 hr exposure to MIC at 3-ppm induced lesions of the nasal cavity and lung lesions.

After 6 days inhalation of 30 minutes of MIC at 0.212 ppm, rats had decreased body weight gain

After a 2x4 day inhalation (6 hr/day), rats presented microscopic lesions in the respiratory tract.

The lowest relevant NOAEL = 0.6 ppm (0.335 mg/kg bw/d) for MIC could be considered for deriving a chronic reference exposure level based on the decreased weight gain and lung pathology immediately after cessation of exposure.

Table B.6.8.1.1-4: summary of experimental results reported in the open literature for MIC.

Single dose inhalation toxicity, 3, or 10 ppm, rat	Fibrosis of lung at 10 ppm NOAEL= 3 ppm
Single dose inhalation toxicity, 3, 10 or 30 ppm, mice	NOAEL < 3 ppm
4 day inhalation, 1 or 3 ppm, rat and mice	NOAEL = 1 ppm
6 days inhalation, 0.212, 0.265, and 0.349 ppm, rats	NOAEL < 0.212 ppm
2x4 days inhalation exposure, 0.15, 0.6 or 3.1 ppm, rats	NOAEL = 0.6 ppm MIC (0.335 mg/kg bw)

Hydrogen sulfide

According to the California Environmental Protection Agency, 2004, Metam sodium risk characterization document:

H₂S is formed as part of the same monomolecular cleavage reaction that produces MITC from metam sodium under dilute aqueous conditions. H₂S would presumably be produced upon application of metam sodium in an agricultural setting as an adjunct to MITC evolution. Sulfur dioxide and sulfuric acid may be formed when atmospheric H₂S is oxidized.

H₂S disrupts intracellular electron transport by inhibiting cytochrome oxidase. Metabolic acidosis results when the shift from aerobic to anaerobic metabolism occurs, provoking a build-up of lactate. H₂S is also a mucus membrane and respiratory irritant. Death results from respiratory arrest and hypoxia (Ellenhorn, 1997).

Acute toxicity: H₂S levels between 2.5 and 5 ppm were associated with coughing and throat irritation after 15 minutes (Bhambhani and Singh, 1985; cited in OEHHA, 1999). Exposure to 7 ppm H₂S during moderate physical exercise led to impaired oxygen and lactate uptake in the blood (Bhambhani and Singh, 1991). Olfactory fatigue occurs at 50-100 ppm, with respiratory tract and eye irritation developing at 150-300 ppm (though some ocular effects may be present at 10 ppm), severe systemic toxicity at 500 ppm and cardiovascular arrest and death at 700 ppm and above (Ellenhorn, 1997). Symptoms commonly reported after accidental human exposures include dyspnea, sore throat, coughing, chest pain and signs of pulmonary obstruction. Less common symptoms include pulmonary edema, cyanosis and pneumonia. Severe neurological and cardiovascular effects can be present in those recovering from high-level exposures. Mortality resulting from severe acute exposure situations reportedly ranges between 2.8% and 6 % (Arnold *et al.*, 1985).

The mean 4-hour LC₅₀ in rats was determined to be 440 ppm (Tansy *et al.*, 1981). The 1-hour lethal concentration in mice was 673 ppm (RTECS, 1994). Respiratory arrest and death occurred in dogs at 1000 ppm (Haggard and Henderson, 1922). A NOAEL of 10 ppm, based on depression of lung mitochondrial cytochrome c oxidase at 50 ppm was established in a study with Fischer 344 rats (Khan *et al.*, 1990).

Sub chronic and chronic toxicity; effects on rats and mice of H₂S exposure 6 hours/day, 5 days/week, for 90 days at 0, 10.1, 30.5 or 80 ppm (cited in OEHHA, 2000). Weight decrements were observed at the high dose in both sexes. The only other effect was inflammation of the nasal mucosa of the anterior segment of the nose in mice, also at the high dose. The NOAEL was set at 30.5 ppm based on the latter effect. Hulbert *et al.* (1989; cited in Alexeeff *et al.*, 1992) noted moderate to severe dose-related proliferation of ciliated and basal cells and decreased non-ciliated Clara cell numbers in Fischer 344 rats following exposure to 10 and 100 ppm H₂S for 8 hours/day, 5 days/week, 5 weeks. Other pulmonary effects, including lymphocytic infiltrates, pulmonary edema and tracheitis, were also observed. Mild acute suppurative tracheitis, laryngitis and mild chronic nephritis were noted in guinea pigs exposed to 220 ppm H₂S for 7 days (Renne *et al.*, 1980; cited in Alexeeff *et al.*, 1992).

H₂S appeared weakly mutagenic in one *Salmonella* study. No long-term carcinogenicity studies have been conducted. Two reproduction studies in rats did not indicate adverse effects, though there was a dose-dependent increase in delivery time (cited in Alexeeff *et al.*, 1992).

Measurements of H₂S after applications of metam sodium showed levels reaching 76 ppb at 1-4 hours post application, becoming not detectable at 5-7 hours and rising again to 8 ppb at 21-24 hours.

Conclusion:

The lowest NOAEL was reported in the rat study, after 8 hours/day, 5 days/week, 5 weeks exposure where a NOAEL = 10 ppm was reported based on effects on Clara cells. NOAEL = 10 ppm = 15.2 mg/m³ = 3.64 mg/kg bw/d

Carbon disulfide (CS₂)

According to the California Environmental Protection Agency, 2004, Metam sodium risk characterization document:

Human exposure to carbon disulfide (CS₂) may follow metam applications as CS₂ is a degradation product of metam sodium, particularly under acidic conditions (pH<5).

CS₂ is readily absorbed in humans after inhalation exposure. Human and animal studies also indicate absorption of the vapor through skin, and animal studies indicate absorption through the gastrointestinal tract.

Acute toxicity. Acute human exposure to CS₂ by inhalation leads to local irritation, pharyngitis and CNS toxicity. Such exposure becomes life threatening after 30 minutes at 3210-3850 ppm and fatal after 30 minutes at 4815 ppm. Oral exposure to 15 ml is also fatal. Dermal and ocular exposure causes severe burns.

Chronic exposures in humans can result in polyneuritis, encephalopathy, tremors, vertigo, psychosis, myopathy and reductions in nerve fiber conduction velocity. The latter sign was evident after exposure to 1 – 7.6 ppm for an average of ~12 years.

Oral LD50 values: rat, 3188 mg/kg; mouse, 2780 mg/kg; rabbit, 2550 mg/kg; guinea pig, 2125 mg/kg. LC50 (2-hr) values: rat, 25 g/m³; mouse, 10 g/m³.

Subchronic and chronic exposures in animals affect the nervous system, cardiovascular system and kidneys. There is no definitive evidence for CS₂-mediated carcinogenicity in humans or animals, though some possibility remains from epidemiologic studies of exposed populations. Genotoxicity data were not reported.

Increased fetal resorption was reported in rabbits at 25 mg/kg/day (oral) during gestation. Developmental toxicity study: pregnant rats were exposed to 0, 100, 200, 400 or 800 ppm CS₂ for 6 hours/day on gestation days 6-20. Significant reductions in fetal body weight were observed at the LOAEL dose of 400 ppm, establishing the NOAEL at 200 ppm.

Degenerative nervous system changes are also evident in animal studies.

Adverse impacts on the nervous system follow inhalation exposure in both humans and animals. Acute effects in humans include dizziness, fatigue, headache, mood change, lethargy, blurred vision, agitation, delirium, hallucinations, convulsions and coma. Symptomology is evident at as low as 320-390 ppm for several hours.

Measurements of CS₂ after applications of metam sodium showed levels at or below the detection level of 4 ppb (Wofford *et al*, 1994).

Methylamine

Like CS₂, methylamine is produced upon cleavage of metam sodium under acidic conditions.

INERIS reported a summary of toxicity data for methylamine (2003):

CL₅₀ rat = 4400 ppm (5 588 mg/m³).

CL₅₀ mice = 1860 ppm (2400 mg/m³).

Male rats (10/lot) were exposed via nose and mouth, 6 hr /day, 5 day/week for 2 weeks at 75, 250, 750 ppm (95, 317, 952 mg/m³). At 75 ppm, slight nasal irritation was reported. At 250 ppm, all animals had nasal sinus erosion and ulceration. At 750 ppm, RBCs, neutrophils and monocytes, Hb, Ht, ALT, AST, urea and cholesterol were increased. GIT was dilated; spleen and thymus were atrophied, liver necrosis, lung congestion, and nasal mucosa congestion. Nasal sinus necrosis was severe with atrophy and septum perforation.

The oral LD50 in rats is 100 – 200 mg/kg.

It was demonstrated positive in the L5178Y mutagenicity assay, but at several hundred-fold higher concentrations than MIC. Gavage exposure to 122 mg/kg methylamine for 5 days in mice produced a statistically significant 27% drop in white blood cell counts.

A role for methylamine in reproductive/developmental toxicity was investigated. In a mouse study, pregnant dams were exposed to varying doses (intraperitoneal) of methylamine (as well as the di- and trimethyl compounds). Reproductive toxicity was not observed.

According to the California Environmental Protection Agency, 2004 Metam sodium risk characterization document :

This compound is known for its irritant properties to eyes, nose and throat upon brief exposures to 20 – 100 ppm. Severe methylamine exposure may lead to pulmonary edema. A NOAEL could be set at 75 ppm.

Carbonyl sulfide:

Carbonyl sulfide (COS) is produced upon cleavage of MITC in the gut.

Acute Toxicity data:

Inhalation-RAT:LC50 1070 ppm/4h

Intraperitoneal-RAT:LD50 23 mg/ kg

Inhalation –Rabbit: LC50: 2550 mg /m³

- Inhalational Exposure to Carbonyl Sulfide Produces Altered Brainstem Auditory and Somatosensory-Evoked Potentials in Fischer 344N Rats (Herr et al, 2007)

In this paper, a summary of toxicity of COS is reported:

- Acute (4 h) inhalation of 804–1189 ppm COS by Sprague-Dawley rats produced neurological signs such as hypoactivity, tremors, and convulsions at 1062 ppm or greater.

- Nose-only exposure of Fischer 344 rats for up to 4 h to 250–1400 ppm COS. Animals surviving > 500 ppm COS were reported to have a motor impairment, and histopathology showed swelling of myelin sheaths in the cerebellum, corpus callosum, and pyramidal tracts.

- Exposure of Fischer 344 rats to up to 500 ppm COS for 2 weeks produced brain lesions in the frontoparietal cortex, putamen, thalamus, red nucleus, and auditory structures (posterior colliculus and anterior olivary nucleus).

- A 12-week inhalation study in rabbits exposed to 50 ppm COS did not result in clear adverse effects.

- F344 rats were exposed to 75-600 ppm COS 6 h per day, 5 days per week for up to 12 weeks. In rats exposed to 500 or 600 ppm for up to 4 days, malacia and microgliosis were detected in numerous neuroanatomical regions of the brain by conventional optical microscopy and magnetic resonance microscopy (MRM). After a 2-week exposure to 400 ppm, Slight gait abnormality was detected in 50% of the rats and hypotonia was present in all rats exposed to COS. Decreases in motor activity, and forelimb and hindlimb grip strength were also detected. In rats exposed to 400 ppm for 12 weeks, predominant lesions were in the parietal cortex area 1 (necrosis) and posterior colliculus (neuronal loss, microgliosis, hemorrhage), and occasional necrosis was present in the putamen, thalamus, and anterior olivary nucleus. Carbonyl sulfide specifically targeted the auditory after exposure for 2 weeks to 400 ppm COS. A concentration-related decrease in cytochrome oxidase activity was detected in the posterior colliculus and parietal cortex of exposed rats as early as 3 weeks. Cytochrome oxidase activity was significantly decreased at COS concentrations that did not cause detectable lesions, suggesting that disruption of the mitochondrial respiratory chain may precede these brain lesions. Our studies demonstrate that this environmental air contaminant has the potential to cause a wide spectrum of brain lesions that are dependent on the degree and duration of exposure. NOAEL < 75 ppm.

- Fisher 334N rats were exposed to 0, 200, 300, or 400 ppm COS for 6 h/day, 5 days/week for 12 weeks, or to 0, 300, or 400 ppm COS for 2 weeks using whole-body inhalation chambers. After treatment, the animals were studied using neurophysiological tests to examine: peripheral nerve function, somatosensory-evoked potentials (SEPs) (tail/hindlimb and facial cortical regions), brainstem auditory-evoked responses (BAERs), and visual flash-evoked potentials (2-week study). Additionally, the animals exposed for 2 weeks were examined using a functional observational battery (FOB) and response modification audiometry (RMA).

Peripheral nerve function was not altered for any exposure scenario. Likewise, amplitudes of SEPs recorded from the cerebellum were not altered by treatment with COS. In contrast, amplitudes and latencies of SEPs recorded from cortical areas were altered after 12-week exposure to 400 ppm COS. The SEP waveforms were changed largely after forelimb stimulation than tail stimulation in the 2-week study. The most consistent findings were decreased amplitudes of BAER peaks associated with brainstem regions after exposure to 400 ppm COS. Additional BAER peaks were affected after 12 weeks, compared to 2 weeks of treatment, indicating that additional regions of the brainstem were damaged with longer exposures. The changes in BAERs were observed in the absence of altered auditory responsiveness in FOB or RMA. This series of experiments demonstrates that COS produces changes in brainstem auditory and cortical somatosensory neurophysiological responses that correlate with histopathological damage at 400 ppm.

NOAEL= 300 ppm (=780mg/m³) = 187mg/kg bw/d.

Table B.6.8.1.1-5: Conversion of ppm into mg/m³ the different metabolites of metam and estimated NOAELs reported for different metabolites of metam sodium.

Compound	Ppm and mg/m ³	NOAEL
Methylisocyanate	1 ppm = 2.55 mg/m ³	1.53mg/m ³
MITC	1 ppm = 3.26 mg/m ³	5 mg/m ³
Methylamine	1 ppm = 1.27mg/m ³	95 mg/m ³
H ₂ S	1 ppm = 1.52 mg/ m ³	15.2 mg/m ³
COS	1 ppm = 2.6 mg/m ³	780 mg/m ³
CS ₂	1 ppm = 3.3 mg/ m ³	10 g/m ³ (LC50)

B.6.8.1.2 toxicity studies on impurities (Annex II A 5.8.1)

See confidential part of the DAR (Vol 4, Annex C).

B. 6.8.2 Supplementary studies (Annex IIA 5.8.2)**Published information:**

- **Sodium Methyl dithiocarbamate Inhibits MAP Kinase Activation through Toll-like Receptor 4, Alters Cytokine Production by Mouse Peritoneal Macrophages, and Suppresses Innate Immunity (Pruett et al, 2005)**

Findings: the authors of the study stated that initial experiments demonstrated that metam administered by oral gavage substantially decreased IL-12 production and increased IL-10 production induced by lipopolysaccharide in mice. SMD decreased the activation of MAP kinases and AP-1 but not NF- κ B in peritoneal macrophages. The expression of mRNA for IL-1 α , IL-1 β , IL-18, IFN- γ , IL-12 p35, IL-12 p40, and macrophage migration inhibitory factor (MIF) was inhibited by SMD, whereas mRNA for IL-10 was increased. SMD increased the IL-10 concentration in the peritoneal cavity and serum and decreased the concentration of IL-12 p40 in the serum, peritoneal cavity, and intracellularly in peritoneal cells (which are >80% macrophages). Similar effects on LPS-induced cytokine production were observed following dermal administration of SMD. The major breakdown product of SMD, methylisothiocyanate (MITC), caused similar effects on cytokine production at dosages as low as 17 mg/kg, a dosage relevant to human exposure levels associated with agricultural use of SMD. Treatment of mice with SMD decreased survival following challenge with non-pathogenic *Escherichia coli* within 24–48 h, demonstrating suppression of innate immunity.

Conclusion of the RMS:

All experimental results are expressed in figures and it is therefore impossible to have a correct view of experimental results.

Metam sodium and its main metabolite MITC could induce some immunological manifestations.

Material and methods:

Five Female C57 black/6 x C3H mice were used and were treated with bacterial lipopolysaccharide from *E.Coli* by IV injection at 60 μ g/mouse. Metam and MITC were from Sigma Chemical. MITC was dissolved in tissue culture grade water and stored frozen until use. Metam was freshly prepared for each assay. Mice were treated by a single dose by gavage with metam (50, 100, 200, 300 mg/kg bw) or MITC (17, 45 mg/kg) which was given just before LPS.

B.6.8.3 Summary of toxicity studies on metabolites and supplementary studies (Annex IIA 5.8)

In soil, an average of 92% of metam sodium decomposes to MITC within a few hours and depending on soil moisture and soil decomposition, substantial amounts of MITC may remain in the soil for 12 days or more, in spite of the volatility of this compound. Photolysis of metam sodium in water and photolysis of MITC in air have been reported. Products in water include MITC and 1, 3-dimethylthiourea. Product in air includes methyl isocyanate (MIC) methyl isocyanide, sulfur dioxide, hydrogen sulfide, carbonyl sulfide, N-methylformamide and methylamine. Methylisocyanate is more toxic than MITC and it as well as some of these other compounds may contribute to some of the toxic effects of metam sodium.

Thus, while metam sodium is itself non-volatile, its application results in significant production and emission of air contaminants. The half-life of MITC in air was reported as 29 to 39 hours in natural sunlight.

Therefore, following agricultural metam-sodium applications, inhalation exposure is essentially related to MITC, but might also include other degradation products such as CS₂, H₂S and MIC. There is uncertainty about how these breakdown products interact to produce the overall potential toxicity deriving from the use of metam-sodium, but MITC, MIC and H₂S have all been associated with ocular and respiratory irritation. While there are no data to address mixed exposure effects, additive or synergistic effects of MITC, MIC and H₂S in respiratory and ocular tissues are plausible.

A quite complete dossier related to **MITC** is included in the DAR. For MIC, the company did not provide studies. Therefore, RMS used data reported in the open literature. ECB (Ispra) reported a rationale for classification of methylisocyanate as T; R24/R25 for dermal and oral toxicity, T; R26 for inhalation and respiratory tract irritation (R37). MIC is a skin irritant (Xi, R38) and an eye irritant (R41). MIC is highly toxic for humans and an accidental release in Bhopal (India) caused thousands of deaths by acute respiratory failure. Respiratory toxicity was also seen in animal studies. MIC was classified by ECB as T⁺; R26, T; R24/25, Xn; R42/43, Xi; R37/38/41. H₂S is a respiratory tract irritant and symptoms commonly reported after accidental human exposure include dyspnea, sore throat, coughing, chest pain and signs of pulmonary obstruction. Acute human exposure to CS₂ by inhalation leads to local irritation, pharyngitis and CNS toxicity. Methylamine is known for its irritating properties to eyes; nose and throat upon brief exposure, and severe exposure may lead to pulmonary edema. COS toxicity was investigated in laboratory animals and CNS lesions were reported.

The company addressed the possible presence of MITC, MIC, CS₂ and methylamine under field conditions at toxicologically meaningful concentrations after metam sodium applications. As reported under point B.6.15.2 in the study of Links, 2006, levels of CS₂, methylamine, and MIC at day 0-4, after application of metam sodium at realistic application conditions, were <0.4 mg/m³, 0.027 mg/m³, and <0.004 mg/m³, respectively. From this study, it was concluded that the potential exposure of the operator to MIC and the stationary measurements to MIC inside the tractor were both below the limit of detection. MIC, CS₂ and methylamine were not detected around the treated field. At detection limit, the upper bound estimate for results of MIC for all field samples would be 4-µg/m³. For CS₂ this would be 400-µg/m³ and for methylamine, this would be 27-µg/m³. Air concentrations of MITC ranged from the limit of detection (0.042 µg/m³) to a maximum concentration of 36-µg/m³ with a GM of 0.9 µg/m³ (n=96). The 95th percentile was 13.2 µg/m³. MITC concentrations in the tractor cabin were at the level of 0.070-0.076 mg/m³ during application reaching 0.107 mg/m³ outside the tractor. These results show that under realistic application conditions of metam sodium, while MIC, CS₂ and methylamine were below the limit of detection, MITC was identified at significant levels, confirming the need to assess operator exposure to MITC but not to MIC or other metabolites. The toxicity of MITC was investigated by the company and a full dossier is included and reported in parallel to metam sodium.

B.6.9 Medical data (Annex IIA 5.9)

B.6.9.1 Report on medical surveillance on manufacturing plant personnel (Annex IIA 5.9.1)

No medicinal surveillance data for manufacturing plant personnel was found for metam sodium.

B.6.9.2 Report on clinical cases and poisoning incidents (Annex IIA 5.9.2)

Sodium methylthiocarbamate (SDM) is the third most abundantly used conventional pesticide in the U.S. In 1991, a train derailment near Dunsmuir, CA, caused a spill of 19,000 gallons (= 71 922 L) of a 32% solution of metam. Over 700 persons in the area sought medical attention for symptoms ranging from nausea and dizziness to irritation of the eyes and upper respiratory tract. A number of persons reported exacerbation or induction of asthma following exposure. Major exposure events have also occurred after routine agricultural use of SMD, leading to the evacuation of schools and of a large portion of the town of Earlimart, CA. In addition, >90,000 people in California are exposed each year to doses of the major breakdown product of SMD, methylisothiocyanate (MITC), in excess of safe dose levels. Considering that only about 20% of total SMD use in the U.S. occurs in California, the actual number of exposures is almost certainly larger. The Reference doses for SMD and MITC are based on rodent studies and application of uncertainty factors, and they seem to be quite accurate with regard to actual measured effects in human subjects. This is of interest because

the dosages used in the present study encompass the dosages used in rodent studies to determine the human reference dose (Pruett et al, 2005).

A series of illnesses occurred in Earlimart, CA on November 13, 1999 following a metam-sodium sprinkler application to a potato field on the southern edge of the town.

Case information was obtained from direct interviews, from illness complaints filed with state or county agencies, or from illness reports filed by community physicians for 173 subjects. Air concentrations of the by-product methyl isothiocyanate (MITC) during the episode were estimated based upon the Industrial Source Complex Short-Term (ISC3) air dispersion model, estimates from prior metam-sodium monitoring studies, and data from weather stations in two adjoining communities.

Weather station data indicated that the November 13th incident corresponded with a temperature inversion at approximately 5:00 p.m. and a shift in the direction of prevailing wind from northwest to southeast. On the edge of Earlimart, 1-hr time weighted average (TWA) MITC concentration estimates ranged from 0.5 to 1 ppm. Of the 173 subjects, 170 had MITC-compatible symptoms, including eye or upper respiratory irritation (77.6%), non-specific systemic symptoms (64.7%), and lower respiratory symptoms (20.0%). 78.2% were exposed within 0.5 miles of the northern edge of the treated field where average 1 hr MITC concentrations exceeded the 800 ppb ocular irritation threshold. (O'Malley et al., 2004).

Bowler et al. (1994) studied the psychological and psychophysiological sequel in a community 3-4 months after a railroad spill of 19,000 gallons of the pesticide metam sodium in the Upper Sacramento River in northern California. No monitoring was done until several days after the spill. The symptoms of 705 people that reported health problems at medical centers during the first month post-spill included headache (64%), nausea (46%), eye irritation/blurring (40%), dizziness (30%), shortness of breath (27%) and diarrhea (25%). Complaints of depression, disorientation, drowsiness, dry mouth, earache, fatigue, fever, hot flashes, irritability, memory reduction, nosebleed, numbness, pain in the arms or legs, tinnitus (ringing in the ear) and sweating were also reported at medical centers. Physiological measures were blood pressure, pulse and cortisol level. Sixty-one percent of the spill residents showed clinical abnormalities. The spill residents had significantly more worried and lower perceived social support than their matched controls. Significantly higher blood pressure and less fluctuation of salivary cortisol levels were also found. More neurological, memory and concentration, anxiety, depression, sleep disorders, headaches, visual, olfactory, dermatological, gastro-intestinal and cardiac symptoms were reported than the controls. Comparing those who smelled the substance with those who did not, increased impact of the event, greater levels of intrusion and avoidance, more affective disturbance and increased diastolic blood pressure was found. In a subgroup of matched pairs (age, education, gender, race and number of children), greater levels of depression, anxiety, and somatic symptoms in the spill residents, greater environmental worry and lower perceived social support were found.

B.6.9.3 Observations on exposure of the general population and epidemiological studies (Annex IIA 5.9.3)

- **Illnesses related to shank application of metam-sodium, Arvin, California, July 2002 (O'Malley M et al, 2005):**

The purpose of the study was the evaluation of the health effects of methyl isothiocyanate (MITC) and other byproducts resulting from the soil-incorporated (shank) application of 25,000 pounds of metam-sodium on July 8, 2002, near the community of Arvin, California. Residents in a four-block area were interviewed regarding eye and upper respiratory irritation, non-specific systemic symptoms, and lower respiratory complaints. The distribution of cases was compared to results of Industrial Source Complex (ISC3) air dispersion modelling for the metam-sodium by-product, methyl isothiocyanate (MITC). The 1-hour 200 ppb no-observed-effect-level (NOEL) and 800 ppb lowest-observed-effect level (LOEL) from a previous human eye irritation study were used to interpret the results of the air modelling estimates. Peak concentrations were compared to the 4-minute NOEL of 600 ppb and the LOEL of 1.9 ppm.

Two-hundred-fifty-two cases of irritant, non-specific systemic and respiratory symptoms were associated with the metam-sodium application. These included 178 community residents or visitors and 74 employees of a carrot packing operation located in the affected neighbourhood of Arvin. The most severe reported illness occurred in a community visitor with a history of pre-existing pulmonary disease, who was hospitalized for a week with respiratory distress. ISC3 Modelling indicated 1-hour MITC concentrations in the affected community ranged from 0.8-1.0 ppm, in the range of the LOEL, with peak concentrations between 2.4 and 3.2 ppm.

Estimated MITC concentrations during the episode exceeded both the 4-minute NOEL and 1-hour NOEL by approximately four fold. The high concentrations of MITC present in the affected neighbourhood may have been partially attributable to failure to immediately complete a required post-application water-treatment on 15 of the 100 treated acres. However, because of the limited area involved, the violation was unlikely to have accounted for the entire incident. Similar episodes may occur when metam-sodium fumigants are used adjacent to other rural communities.

- **Irritant dermatitis among workers cleaning up a pesticide spill: California 1991 (Koo et al., 1995) :**

An outbreak of dermatitis occurred among county jail inmates who removed dead fish from the Sacramento River in California after a spill of metam sodium. The spilled chemical decomposes to methylisothiocyanate (MITC), a known skin irritant. A retrospective cohort study was conducted among the inmates and their crew leaders. Among 42 jail group members, 27 had dermatitis involving the feet and ankles; dermatitis was associated with lower extremity water contact (RR = 3.4, 95% CI 1.0-11.8); the attack rate increased with length of time spent in the water. For comparison, other state and federal employees who worked in the river at the same time were also interviewed. None reported dermatitis. Over three-quarters (24/31) of these other clean-up workers whose feet became wet changed to dry clothing immediately; none of the jail group changed immediately. The river concentration of MITC measured 20-40 ppb at the time of exposure. We speculate that prolonged wetness, occlusive boots, friction, and heat contributed to chemical irritation at this low concentration; the experience of the other clean-up workers suggests that this outbreak could have been prevented.

- **Persistent respiratory health effects after a metam sodium pesticide spill (Cone et al., 1994)**

Report on the occurrence of persistent respiratory disorders, including irritant-induced asthma, among adults living and working near an environmental spill of the pesticide, metam sodium, after the derailment of a tank car. DESIGN: Retrospective clinical case series. SETTING: California communities situated within one-half mile of the Sacramento River, from Mt. Shasta City to Shasta Lake. PATIENTS: 197 adults referred to a university occupational/environmental health clinic or to a private occupational/environmental health practitioner for evaluation of health problems potentially related to the spill. MEASUREMENTS AND RESULTS: History, physical examination, review of medical records, spirometry, and methacholine challenge testing revealed 20 cases of persistent irritant-induced asthma and 10 cases of persistent exacerbation of asthma. CONCLUSIONS: This is the first reported series of cases of persistent irritant-induced asthma involving both community residents and occupationally exposed individuals

B.6.9.4 Clinical signs and symptoms of poisoning and details of clinical tests (Annex IIA 5.9.5)

Based on data from animal studies with toxic doses, the signs of toxicity are likely to be lachrimation, blepharospasm, conjunctivitis and chemical burns after contact with eyes, primary irritation, dermatitis, eczema, chemical burns after contact with skin, nausea, vomiting, burns, gastroenteritis, sleepiness, cardiac and respiratory disorders, shock, renal lesions after ingestion, and primary mucosal and upper respiratory tract irritation, throat pain, cough hoarseness after inhalation.

B.6.9.5 First aid measures – Therapeutic regimes (Annex IIA 5.9.5)

Ingestion: give large quantities of water to drink. Do not induce vomiting and treat symptomatically. No specific antidotes known.

Eye contact: rinse immediately with plenty water for at least 15 minutes and seek medical advice.

Skin contact: remove contaminated clothing immediately and wash skin with soap and water. Rinse with more water or shower and seek medical attention.

Inhalation: remove patient from exposure to the product and keep well ventilated with fresh air. Keep at rest and if necessary, give artificial respiration. Seek medical attention.

Medical treatment: this compound is considered harmful if swallowed and if inhaled. Metam sodium belongs to the group of dithiocarbamates. There is no specific antidote. Supportive care should be given and the medical treatment should be based on the judgment of the physician in response to the reaction of the patient.

B.6.9.6.1 Expected effects and duration of poisoning as a function of the type, level and duration of exposure or ingestion (Annex IIA 5.9.6)

The prognosis for the expected effects of acute exposure through inhalation of metam sodium and MITC is that it results in respiratory irritation, eye irritation and signs of gastrointestinal complaints.

B.6.9.6.2 Expected effects and duration of poisoning as a function of varying time periods between exposure or ingestion and commencement of treatment (Annex IIA 5.9.6)

The prognosis for the expected effects of acute exposure through inhalation of metam sodium and MITC is that it results in respiratory irritation, eye irritation and signs of gastrointestinal complaints.

WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Registration must not be granted on the basis of this document.

B.6.10 Summary of mammalian toxicology and proposed ADI, AOEL and drinking water limit (Annex IIA 5.10)

B.6.10.1 Summary of mammalian toxicology:

Absorption, distribution, metabolism and excretion:

Oral absorption of metam and its main metabolite MITC is rapid and important reaching a mean value of 85% and 90% of metam or MITC, respectively. Comparing the results from the high and low dose levels, it is clear that metabolism of metam sodium may be saturated at high dose levels resulting in a reduced breakdown of MITC, which is then excreted unchanged via exhaled air.

Tissue distribution is important and suggests that thyroid could be a target organ. Approximately 2.3% of the dose was still detected at 168 h in tissues. Half-life of elimination from plasma ranged from 60.8 to 74.1 hours. Metabolism is extensive and rapid, suggesting a decomposition of metam into MITC, CO₂, and COS. MITC is further conjugated to GSH and excreted in urine while CO₂ and COS are excreted via expired air. The other significant pathway for metam is the release of CS₂, which could be related to the acidic conditions existent in the stomach of the rat (pH3.8-5) following oral ingestion.

From published studies in the open literature, it appears that, although similar metabolites were identified in rats and mice, non identified part of polar metabolites are more important in mice than in rats suggesting that metabolism of metam and MITC after ip administration, is quantitatively and qualitatively different.

The majority of the material given was excreted within 24-48 h after administration with minor portions excreted up to 168 h after dosing.

Acute toxicity studies:

Metam breaks down to MITC, which is the ultimate toxicant. The gaseous decomposition of metam sodium into MITC under acidic conditions justifies the need for a R31.

Acute oral exposure to metam or MITC led to pilo-erection, hunched posture, abnormal gait, lethargy, decreased respiration rate, ptosis, and pallor of extremities, increased salivation, and clonic convulsions. Metam and MITC should be classified as harmful or toxic by ingestion, respectively.

After dermal exposure, hypothermia was reported after metam sodium while quite similar signs as those reported after oral exposure was seen after MITC dermal exposure. MITC should be classified as harmful by skin exposure.

After whole body inhalation exposure to metam, signs consistent with exposure to an irritant and toxic aerosol were noted. Histopathology of the lungs showed bronchiolar epithelial necrosis with inflammatory exudates. Classification as harmful by inhalation and R20 is required according to this study. A nose and head only inhalation exposure was realized where reddish discharges from nose and eyes were reported and animals had high stepping gait. According to the results of this study, metam should not be classified for inhalation toxicity. Clinical signs reported after exposure to MITC by inhalation were quite similar to those seen after metam. MITC requires a classification as toxic by inhalation (whole body inhalation study).

Metam and MITC were corrosive in the skin irritation test. No irritation effects were seen in the eye irritation test. Skin sensitization potential was seen for metam sodium and for MITC.

Table B.6.10.1-1: Summary of acute toxicity of Metam sodium

Type of test Test species	Test substance purity	Results	References
Acute oral, rat	Batch n°? 510 g/L	LD ₅₀ combined: 896 mg/kg bw harmful Xn, R22	Baldrick and Healing, 1991a
Acute dermal, rat	Batch n°? 510 g/L	LD ₅₀ >2000 mg/kg bw	Baldrick and Healing, 1991b
Acute inhalation, rat whole body	Batch n°? 510 g/L	LC ₅₀ combined= 2.54 mg/L Xn, R20	Jackson and Hardy, 1992
Acute inhalation, rat head and nose	42.2%, batch ZH 130585	LC ₅₀ > 6.8 mg/L	Gelbke and Klimisch, 1986
Acute skin irritation, rabbit	Batch n°? 510 g/L	Corrosive : C, R34	Ligget and McRae, 1991a
Acute eye irritation, rabbit	Batch n°? 510 g/L	Non irritant	Ligget and McRae, 1991b
Skin sensitization- M&K test	Batch n°? 510 g/L	Skin sensitiser, R43	Parcell and Danton, 1991

Table B.6.10.1-2: Summary of acute toxicity of Metam potassium

Type of test Test species	Test substance purity	Results	References
Acute oral, rat	batch n° V814/2207KF, 54.7% w/w	LD ₅₀ =1000 mg/kg bw harmful Xn, R22	Sulaiman, 2004
Acute dermal, rat	V814/2207KF, 54.7% w/w	LD ₅₀ > 2000 mg/kg bw	Sulaiman, 2002
Acute inhalation, rat head and nose	batch n° V814/2207KF, 54.7% w/w	LC ₅₀ combined= 3.04 mg/L Xn, R20	Venugopola Rao, 2002
Acute skin irritation, rabbit Metam sodium	Batch n°? 510 g/L	Corrosive : C, R34	Ligget and McRae, 1991a
Acute eye irritation, rabbit	batch n° V814/2207KF, 54.7% w/w	Non irritant	Prakash, 2002
Skin sensitization- M&K test	batch n° V814/2207KF, 54.2% w/w	Not sensitiser	Merkel, 2002

Table B.6.10.1-3: Summary of acute toxicity of MITC

Type of test Test species	Test substance purity	Results	References
Acute oral, rat	MITC; lot n° 6205 MK, 98%	LD ₅₀ combined: 147 mg/kg bw. Toxic T, R25	Kieczka and Kirsch, 1986
Acute dermal, rat	Lot n° 6205 MK, 98%	LD ₅₀ =1290 mg/kg bw Xn, R21	Kirsch and Hildebrand, 1987
Acute inhalation, rat whole body	98%	LC ₅₀ = 0.54 mg/L T, R23 Xi, R37	Jackson et al, 1981
Acute skin irritation, rabbit	Lot n° 6205 MK, 98%	Corrosive : C, R34	Kirsch and Kieczka, 1986
Acute eye irritation study		No study is required.	
Skin sensitization- M&K test	Batch n°,	Skin sensitiser; R43	Kieczka and Kirsch,

Type of test Test species	Test substance purity	Results	References
	6205MK; 98%		1986

Summary of short term-toxicity studies:

Drinking water range finding studies were performed for short periods of exposure in mice (6-8 days) and in rats (21 days). The studies were used to set dose levels for the subsequent 90 -day studies.

Rats and mice received metam sodium in drinking water for a 90-day period. Due to the instability of metam sodium in water, the doses of metam sodium actually received were recalculated assuming maximum degradation. In rats, at 18-20 mg/kg bw/d, the olfactory epithelium in the posterior nasal passage was affected and Bowman's glands were prominent and/or vacuolated and disorganized. The NOAEL was set at 0.79 mg/kg bw/d. In mice, at 36-38 mg/kg bw/d, mucosal hyperplasia and epithelial eosinophilia was apparent in the urinary bladder. Nasal cavity was not affected. A NOAEL at 0.49 mg/kg bw/d is proposed.

In a 65-day inhalation study, at 45 mg/m³ metam sodium (12 mg/kg bw/d), rats nasal passages presented a mild degree of mucigenic epithelial hyperplasia as well as lymphocytic rhinitis. Pulmonary histiocytosis and gastric erosions of the stomach were seen in both sexes at 160 mg/m³ (42 mg/kg bw/d). The NOAEL was set at 6.5 mg/m³.

In dogs, metam sodium was given in capsules for 90 days and caused treatment related hepatitis at 5 mg/kg bw/d onwards, resulting in the early termination of two dogs in the top dose group due to adverse clinical signs. Treatment-related hepatitis was associated with progressive inappetence and weight loss. There was a variety of changes, secondary to hepatotoxicity or indicative of generalized toxicity or poor clinical conditions. Considerable reversal of both macroscopic and microscopic changes in the liver had taken place between cessation of treatment with metam sodium and termination of the study. After 1-year, hepatotoxicity was more evident in female dogs as suggested by increased ALT and some liver histopathological findings. The NOAEL was set at 0.1 mg/kg bw/d.

Rabbits did not show systemic toxicity after dermal exposure for 21 days to metam sodium. Local skin reactions such as erythema edema and rhagades and epidermis-dermatitis were seen at 62.5 and 125 mg/kg bw/d. NOAEL for local effects = 31.25 mg/kg bw/d.

As severe toxic effects are reported at doses lower than 50 mg/kg bw/d, RMS considers that metam sodium should be classified R48/22.

According to the company, based on the provisions of EU Directive 2001/59/EC (28th ATP) and taking into account the NOAEL derived in the 90-day rat study, it is principally justified to classify metam with R48/22. However, there was no serious damage or even overt toxicity/mortality seen in the 90-day and/or one-year dog studies. The most pronounced effect in the 90-day study was hepatitis and a drastic increase in liver enzyme parameters.

These effects on liver and liver enzyme parameters were shown to be completely reversible with full recovery observable, which was agreed upon in the evaluation of the RMS. In light of the reversibility of effects on liver and liver enzymes, in the absence of other severe systemic effects and in view of the occurrence of effects in an individual animal only (see metam one-year study), a classification with R48/22 is not considered to be appropriate. Effects mediated by local contact at the site of first contact will be mitigated by consideration of suitable protective equipment and are not within the scope of the R48. Such effects are rather addressed by R phrases applicable for local effects (i.e. R34/35-R38/37/38).

A 4-week inhalation rat study was performed with MITC. At doses of 100 mg/m³, lung weight was increased, and was associated with bronchopneumonia and epithelial proliferation in bronchi and bronchiole. At this dose level, proliferation in tracheal epithelial cells and inflammatory changes in the nasal cavity and atrophy of the olfactory epithelium as well as focal metaplasia of squamous epithelial cells in the area of respiratory epithelium were reported. The company proposed a NOAEL = 20 mg/m³ (5.76 mg/kg bw/d). A NOAEL for systemic effects is proposed at 5 mg/m³.

In a 90-day dog study by gavage with MITC, at the dose level of 2 mg/kg bw/d body weight decrease, haematological findings and blood chemistry were observed.

Table B.6.10.1-4: Summary of short term-toxicity of Metam.

Type of test Test species	Test substance purity	Results			References
		NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Symptoms	
90-day drinking water study, mice at 0.018, 0.088, 0.35, 0.62 mg/ml	ref n° Y06930/007 and Y06930/008, 525.54 g/L	0.018 mg/ml = 0.8 mg/kg bw/d	0.088mg/ml = 4.5 mg/kg bw/d	↓ water consumption, hematological changes, histopathological changes in bladder	Whiles, 1991
Oral 90-day drinking water study in rat at 0.018, 0.089 and 0.443 mg/ml	batch n° Y06930/007 and Y03930/008; 525.54 g/L	0.018 mg/ml = 0.5 mg/kg bw/d	0.089mg/ml = 3.1 mg/kg bw/d	↓ body weight, food and water consumption; nasal histopathological findings at top dose	Allen, 1991
Oral 90-day, gavage study in dog at 1, 5 and 10 mg/kg bw/d	Batch n° BAS 005 00N with CTL ref n° Y06930/008 at 43.15%	1 mg/kg bw/d	5 mg/kg bw/d	Clinical signs of toxicity, hepatitis, urinary bladder cell increased mitosis	Brammer, 1992
Oral 1-year dog study by gavage at 0.05, 0.1 or 1 mg/kg bw/d	batch n° BAS/005/00N 90-2; CTL n° Y06930/008; 525.54 g/L (43.148% w/w)	0.1 mg/kg bw/d	1 mg/kg bw/d	Hepatotoxicity (blood chemistry and histopathological findings)	Brammer, 1993
Inhalation 90-day rat study at 6.5, 45, 160 mg/m ³	Vapam technical: lot n° EHC 0355-21; containing 32.7% metam sodium.	Metam: 6.5 mg/m ³ = 1.75 mg/kg bw/d	45 mg/m ³ = 12 mg/kg bw/d	Liver and nasal toxicity	Knapp, 1983
Percutaneous 21 day rabbit study at 31.25, 62.5 or 125 mg/kg bw/d	BAS 00500 N 42.4%	NOAEL local effects = 31.25 mg/kg bw/d NOAEL systemic effects	62.5 mg/kg bw/d >125 mg/kg bw/d	Local dermal effects: erythema, edema, rhagades; epidermodermatitis No systemic toxicity.	Leuschner, 1979

Table B.6.10.1-5: Summary of short term-toxicity of MITC

Type of test Test species	Test substance purity	Results			References
		NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Symptoms	
MITC: 4-week inhalation study in rats at 5, 20, 100 mg/m ³	Batch n° 6205MK, 96.9%	5 mg/m ³ (1.7 ppm)	20 mg/m ³ = 6.8 ppm	Nasal cavity atrophy	Klimisch, 1987
MITC: 90-day dog study gavage at 0.04, 0.4 or 2 mg/kg bw/d	Batch n° BX340178 AD11308, 95.96% and batch n° BX 340178 AD11328 at 95.96% formulation CR 20593/I, AD11331	0.4 mg/kg bw/d	2 mg/kg bw/d	body weight decrease, haematological findings and blood chemistry	Harling et al, 1986

Summary of genotoxicity

Metam-sodium was evaluated for possible mutagenic/genotoxic effects in *in vitro* test systems using bacterial and mammalian cells and in *in vivo* test systems using hamsters and mice.

In the bacterial revertant assay, employing the standard plate test as well as the preincubation test method Metam-sodium was negative in the presence or absence of metabolic activation towards *S. typhimurium* strains TA 92, TA 98, TA 100, TA 1535, TA 1537 and TA 1538.

In the HGPRT assay in CHO cells, slight increases in mutation rates cannot be ruled out completely although the results are not dose-related.

Metam-sodium revealed a slight clastogenic activity in the presence and absence of metabolic activation in the first chromosome aberrations test and in the presence of metabolic activation system in the second test, both performed in human lymphocytes. Increases in structural chromosomal aberrations were observed at concentrations that had cytotoxic effects on chromosomal morphology which prevented metaphases from accurate analysis for structural chromosomal aberrations.

In the *in vitro* UDS assay to assess DNA damage in freshly isolated primary rat hepatocytes metam-sodium did not induce unscheduled DNA synthesis even at concentration being cytotoxic as demonstrated by the net nuclear grain count and the percentage of nuclei with more than 6 or 20 grains.

Metam-sodium did not induce micronuclei in bone marrow polychromatic erythrocytes in male and female CD-1 mice and does not possess clastogenic activity in the mouse bone marrow micronucleus test.

In the *in vivo* chromosome aberration test performed in Chinese hamsters, Metam-sodium is not clastogenic.

In conclusion, Metam-sodium induced equivocal results in *in vitro* tests evaluating mammalian gene mutations and chromosomal aberrations.

Metam-sodium did not induce unscheduled DNA synthesis in primary hepatocytes. *In vivo* cytogenic investigations were negative. Overall, it can be concluded that metam sodium is not genotoxic *in vivo* (confirmed during the Peer Review).

Table B.6.10.1-6: Summary of genotoxicity of Metam

Type of test Cell/Test species	Conditions	Test substance purity	Results	References
In vitro tests				
<i>Salmonella typhimurium</i> strains TA 92, TA 98, TA 100, TA 1535, TA 1537 and TA 1538	Standard plate test: 20 – 5,000 µg/plate (+ S9/-S9) Preincubation test: 4 – 5,000 µg/plate (+ S9/-S9)	Batch n° ZH130585; 42.2%	Negative	Engelhardt, (1987)
CHO cells (HGPRT) locus	0.0000464 - 0.01mg/mL (+/-S9)	Batch n° ZH130585; 42.2%	Equivocal	Engelhardt, (1987)
CA in human lymphocytes	+ S9: 10 – 40 µg/mL - S9: 1 – 20 µg/mL	Batch n° ZH130585; 42.2%	Positive	Gelbke and Engelhardt, (1987)
CA in human lymphocytes	+ S9: 5 – 40 µg/mL - S9: 2.5 – 30 µg/mL	Batch n° WCR 14989-13-01 ; 32.3%	Equivocal	Mackay, (1996)
<i>in vitro</i> UDS	primary rat hepatocytes 0.5 – 250 nL/mL	Batch n° ZH130585; 42.2%	Negative	Cifone, (1987)
In vivo tests				
Micronucleus test Bone marrow cells CD1-mice	single oral dose males and females: 500 mg/kg bw bone marrow sampling 24 and 48 h after dosing	Batch n° WCR 14989-13-01 ; 32.3%	Negative	Barber and Mackay (1996)
Cytogenetic study <i>in vivo</i> Bone marrow cells of male and female Chinese hamsters	single oral dose (gavage) males and females: 0, 150, 300 and 600 mg/kg bw bone marrow sampling 6, 24 and 48 h after dosing	Batch n° ZH130585; 42.2%	Negative	Gelbke and Engelhardt (1987)

MITC was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 in the presence and absence of metabolic activation.

MITC was further negative in recombination assays in *Bacillus subtilis* strains H17 and M45 with and without metabolic activation.

MITC has no chromosomal damaging effects in an *in vitro* test using human lymphocytes.

In the *in vivo* mouse micronucleus test, MITC did not increase the number of micronucleated polychromatic erythrocytes.

MITC is not genotoxic. From open literature, it was reported that MITC was not genotoxic.

Table B.6.10.1-7: Summary of genotoxicity of MITC

Type of test Cell/Test species	Conditions	Test substance purity	Results	References
in vitro tests				
<i>Salmonella typhimurium</i> strains TA98, TA100, TA1535 and TA1537	+ S9: 20 – 5,000 µg/plate - S9: 20 – 5,000 µg/plate	Batch n° 6205 MK. 98.1%	Negative	Engelhardt, and Gelbke, 1986
Recombination assays with <i>Bacillus subtilis</i> H17 (rec+) and M45 (rec-)	+ S9: 1 – 10,000 µg/plate - S9: 1 – 10,000 µg/plate	Id n° 85/231 charge 6205 MK; 98.1% ?	Negative	Jagannath, 1989
CA in human lymphocytes	+ S9: 0.05, 0.1, 0.5 µg/mL - S9: 0.1, 0.5, 1.0 µg/mL	Batch n° 6205 MK. 98.1%	Negative	Engelhardt and Gelbke, 1987
in vivo tests				
Micronucleus test Bone marrow cells CD1 Swiss mice	single oral dose males and females: 110 mg/kg bw bone marrow sampling 24, 48 and 72 h after dosing	Batch n° 340178 B19 and batch n° 340173 B23 at 95.86%	Negative	Allen et al (1985)

Summary of long-term studies:

Metam sodium was administered to rats in drinking water for 2 years. At 0.19 mg/ml, rats showed signs of systemic toxicity indicated by reduced bodyweight gain, specific lesion within the nasal passages, and changes in some hematology and spleen (hemosiderin depots) parameters and a slight increase in the severity but not of incidence of degenerative myopathy of the voluntary muscle. There were a few changes associated with the 0.056 mg/ml dose level, which were consistent with, but less marked than effects at the high dose. The incidence of hemangiosarcoma in males in the mesenteric lymph nodes was slightly higher than the control at the intermediate dose of 0.056 mg/ml but this effect was not observed at top dose. In view of the overall results obtained in this carcinogenicity study, it can be concluded that metam sodium is not carcinogenic in rats. A NOAEL was established at 0.019 mg/ml.

Mice received in their drinking water metam sodium at 0.019, 0.074 or 0.23 mg/mL for 24 months.

Both male and female mice had urinary bladder lesions and pathological significant changes were mainly present at 0.23 mg/mL. At 0.074 and 0.23 mg/mL, there was an increased incidence of angiosarcoma in males and females. Metam sodium is carcinogenic in mice and the symbol **Xn** and risk phrase **R40** is proposed (limited evidence of a carcinogenic effect). A NOAEL systemic toxicity and carcinogenicity = 0.019 mg/mL is proposed taking into account the increased incidence of urinary bladder epithelial hyperplasia, hepatocytic necrosis and increased incidence of angiosarcoma at 0.074 mg/mL.

The increased incidence of bladder neoplasia reported at top dose is associated with the treatment of metam sodium. The reason for this increase could be related to the induction of chronic hyperplasia in this organ. The extremely low incidence of neoplasia, however, indicates that the transition from hyperplasia to neoplasia is a rather rare event.

Rats received MITC via drinking water at 2, 10 or 50 ppm over 104 weeks. Reduced body weight of male rats, was more important at top dose at the end of the study period, white blood cell parameters, histopathological findings such as bone marrow hyperplasia, increased kidney microcalculi, liver effects, and spleen hyperplasia/increased haematopoiesis were reported at 50 ppm (1.6 mg/kg bw/d) and could be related to MITC exposure. MITC is not carcinogenic under these experimental conditions. A NOAEL = 10 ppm (0.44 mg/kg bw/d) is proposed.

Mice received MITC in drinking water at 5, 20, 80 and 200 ppm. A NOAEL can be set at 20 ppm (3.3 mg/kg bw/d) taking into account the different slight effects seen at 80 ppm (12 mg/kg bw/d) such as the increased incidence of clinical signs, slight decreased body weight and body weight gain, slight effects in blood and altered organ weights. MITC is not carcinogenic in mice.

Table B.6.10.1-8: Summary of long-term toxicity and carcinogenicity of Metam

Type of test Test species	Test substance purity	Results			References
		NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Symptoms	
Rat, 104 week drinking water study at 0.019, 0.056 and 0.19 mg/ml	batch n° BAS/005/OON 90-2; 525.54 g/L = 43.148% w/w	0.019 mg/ml = 1.5 mg/kg bw/d	0.056 mg/ml = 4.3 mg/kg bw/d	nasal passages toxicity, ↓ body weight, changes in some hematological parameters	Allen, 1991 Rattray, 1994
Mice study at 0.019, 0.074 or 0.23 mg/ml in drinking water for 24 months	Batch n° BAS/005/00N 90*2; 525.54 g/L (43.148%)	0.019 mg/ml (1.9 mg/kg bw/d)	0.074 mg/ml (7.2 mg/kg bw/d)	Urinary bladder hyperplasia, ↑incidence of angiosarcoma, and at top dose: urinary bladder neoplasm	Horner, 1994 Pistel, 1994 Xn, R40

Table B.6.10.1-9: Summary of long-term toxicity and carcinogenicity of MITC

Type of test Test species	Test substance purity	Results			References
		NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Symptoms	
104 weeks rat study at 2, 10 or 50 ppm in drinking water	batch n° 28 166: 95.58%; batch n° 29 482: 95.41%; batch n° 29 482: 95.36% and batch n° 29 482: 96.06%	10 ppm (0.44 mg/kg bw/d)	50 ppm (1.6 mg/kg bw/d)	↓ body weight, altered WBC parameters, bone marrow hyperplasia, ↑kidney microcalculi, liver effects, and spleen hyperplasia/increased haematopoiesis	Brown, 1984 Brown, 1988 Iwan, Kaiser, and Hollihn, 1988
Mice, drinking water study at 5, 20, 80 and 200ppm	lot n° MS 25206; 93.14%	20 ppm (3.3 mg/kg bw/d)	80 ppm (12 mg/kg bw/d)	↑clinical signs, ↓body weight, body weight gain, slight effects in blood and altered organ weights	Satoh et al, 1980 Shigeshi Toyoshima, 1982 Kashima and Sato, 1985 Hiraide, 1988

Summary of reproduction toxicity studies:

In rats, metam sodium was tested in a multigenerational drinking water study corresponding to 1.4-216 mg/kg bw/d.

At top dose, body weight was decreased on occasion in males and in females during the different phases of the study. Food consumption was reduced. In females, minimal to marked Bowman's gland duct hypertrophy with loss of alveolar cells was detected in the olfactory mucosa lining the nasal septum and turbinate bones at all levels of the nasal cavity. The changes in the Bowman's gland were accompanied by degeneration/disorganization/atrophy of the olfactory epithelium. The nasal lesion was a direct consequence of the increased dose taken by females during lactation. Nasal passage of offspring of either generation at any dose level was not affected.

The percentage of pups live born was in excess of 94% for each group in both generations. There were no litter losses. There was a reduction in individual pup weight and in total litter weight and a reduction of pup weight gain at top dose in both generations. One female pup of top dose had no ocular issues. Bilateral anophthalmia seems to occur spontaneously in the Alpk: APfSD rat and this isolated incidence in one generation only are considered to be of no toxicological significance.

Developmental studies were performed in rats by gavage at doses ranging from 5 to 120 mg/kg bw/d.

Maternal toxicity started at 10 mg/kg bw/d as suggested by the reduced body weight gain going to a reduced body weight when the dose increases. Reproduction parameters were not affected. A significantly increased post-implantation loss was reported at 120 mg/kg bw/d. At 60 mg/kg bw/d, one female had complete resorption. At 120 mg/kg bw/d, the number of live fetuses was reduced and mean fetal weight was significantly lower.

Fetotoxicity was evident at 80 mg/kg bw/d with a reduced mean fetal weight. At 40 mg/kg, bw/d there was also some evidence for fetotoxicity.

Major defects were reported at 120 mg/kg bw/d, two fetuses/litter exhibited a meningocele (neural tube closure defect) and another fetus had bilateral microphthalmia. At 80 mg/kg bw/d, one fetus/1 litter had a meningocele.

At 60 mg/kg bw/d 5 fetuses/5 litters were affected: defects of eyes (3 fetuses with other head defects); 1 fetus had a shortened jaw and cleft lip, 1 had meningocele, 1 fetus had unossified 2nd, 3rd and 4th arches of the cervical vertebrae, and one fetus displayed an abnormal zygomatic arch. None of which has been seen historically in controls in the laboratory. At 20 mg/kg, one fetus had four major defects, including a shortened lower jaw, which has not been seen in historical data. At 5 mg/kg bw/d, two fetuses in the 5 mg/kg group had abnormal zygomatic arch. Whilst this defect was not seen at 20 mg/kg, a single incidence was seen at 60 mg/kg.

The incidence of specific major defects and number of fetuses affected at 5 and 20 mg was small, not statistically significant, and did not form part of any dose-response relationship, although the possibility of a treatment-related effect cannot be completely dismissed.

Skeletal anomalies and variations were detected in the vertebral column and in sternum and the number affected litters were increased as compared to the control and sometimes higher at 40 mg/kg bw/d than at 120 mg/kg bw/d. These effects were assessed as sign of immaturity of fetuses. There was a dose-related effect for retardations. Retarded ossification of fetal skeletons, especially the incomplete ossification of skull bones could be connected with the mechanism of development of meningocele and influence of metam sodium cannot be excluded.

In rabbits, metam sodium was given by gavage at doses ranging from five up to 100 mg/kg bw/d.

Body weight gain of dams at 100 mg/kg bw/d was lower on most days of treatment. The influence on body weight gain partly corresponds with the diminished feed consumption. At 60 mg/kg bw/d, dams lost considerable weight after starting of dosing and food consumption was reduced. Similar but less marked changes were seen in the 20 mg/kg bw/d group.

At 100mg/kg bw/d, the number of dead implants was increased, especially due to the high number of early resorptions. Increased post implantation loss was seen at 100 and 60 mg/kg bw/d, and reduced number of live fetuses/pregnant female was seen at 30, 60 and 100 mg/kg bw/d. At 60 mg/kg bw/d, fetal weight was decreased. Percentage of male fetuses was reduced and litters containing five or less fetuses did not included males.

Two fetuses in two litters of 100 mg/kg bw/d showed a meningocele or spina bifida. These are rare anomalies in the strain of rabbits used in this study. There was a slight dose-related increase in sternebra asymmetry. The influence of metam sodium cannot be ruled out.

In each of the reported metam sodium studies, single cases of rare anomalies, reaching no statistically significant, were reported at maternal toxic doses and influence of metam sodium cannot be excluded. RMS proposes to classify metam sodium **Repr.Cat 3; R63**.

Similar defects affected head of rat and rabbits and were related to neural tube closure or eyes defects. Fetuses showed retarded ossification in head but also in limbs, thoracic vertebrae, sternum, and these effects were seen at doses with maternal toxicity.

Table B.6.10.1-10: Summary of reproductive toxicity and teratogenicity of Metam

Type of test Test species	Test substance purity	Results			References
		NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Symptoms	
2 generation rat study in drinking water at 0.01, 0.03 or 0.1 mg/ml	batch ref BAS/005/00N; 526 g/L or 43.148%/w; CTL n0 Y06930/008	NOAEL reproduction toxicity >0.1 mg/ml = 12 mg/kg bw/d. NOAEL fetotoxicity= 0.03 mg/ml = 4 mg/kg bw/d NOAEL parental toxicity= 0.03 mg/ml = 4mg/kg bw/d	- 0.1 mg/ml =12.3 mg/kg bw/d 0.1 mg/ml	- ↓ pup and litter weight ↓ body weight during pre-mating, gestation and lactation	Milburn, 1993
Developmental rat study, gavage at 0, 10, 40, 120 mg/kg bw/d day 6-15 gestation	metam sodium batch n° ZH 130585; 42.2% or 517.3 g/L	NOAEL maternal toxicity= 10 mg/kg bw/d NOAEL developmental toxicity=10 mg/kg bw/d	40 mg/kg bw/d 40 mg/kg bw/d	↓body weight, body weight gain, food consumption ↑incidence of variations and retardations at maternal toxic doses; rare anomalies at top dose	Hellwig and Hildebrand, 1987
Developmental rat study, gavage 0, 5, 20 or 60 mg/kg bw/d days 7-16	metam sodium (ref n° BAS/005/00N, CTL n° Y06930/008; 43.15% w/w)	NOAEL parental toxicity= 5 mg/kg bw/d NOAEL developmental tox=5 mg/kg bw/d	20 mg/kg bw/d 20 mg/kg bw/d	- ↓body weight gain, food consumption, piloerection, salivation and urinary incontinence ↓ossification consistent with ↓ fetal weight Major defects were observed at 60 mg/kg bw/d Developmental toxicity: Cat 3, Xn, R 63	Tinston, 1993

Type of test Test species	Test substance purity	Results			References
		NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Symptoms	
Developmental rabbit study, gavage 0, 5, 20 or 60 mg/kg bw/d days 8- 20	metam sodium (ref n° BAS/005/00N, CTL n° Y06930/008; 43.15% w/w)	NOAEL parental toxicity= 5 mg/kg bw/d NOAEL developmental tox=20 mg/kg bw/d	20 mg/kg bw/d 60 mg/kg bw/d	↓body weight gain, food consumption, piloerection, salivation and urinary incontinence ↓ fetal weight; altered sex ratio Major defects were observed at 60 mg/kg bw/d Developmental toxicity: Cat 3, Xn, R 63	Hodge, 1993
Developmental rabbit study, gavage 0, 10, 30 or 100 mg/kg bw/d days 6-18	metam sodium (ref n° 85/232, batch n° ZH130585; 42.2% w/w)	NOAEL parental toxicity= 10mg/kg bw/d NOAEL developmental tox=10 mg/kg bw/d	30 mg/kg bw/d 30 mg/kg bw/d	↓body weight gain ↓ number live fetuses; ↑dead implants Major defects were observed at 100 mg/kg bw/d Developmental toxicity: Cat 3, Xn, R 63	Hellwig, 1987

In a 2 generation rat drinking water study, MITC was given at 2, 10, 50-ppm corresponding to 0.13-5.63 mg/kg bw/d. Variations in weight gain were observed in both sexes at top dose which occasionally attained statistical significance. Gestation and lactation weight were not altered. Mating performance and fertility were not adversely affected. The number of pups born/female was marginally lower at 10 and 50 ppm without reaching statistical significance. Physical and functional development of pups was comparable amongst all groups. Slight delays in the onset of eye opening and pinna unfolding were observed at top dose but were considered incidental to treatment, as there were no delays in the completion of each parameter. Functional development of top dose pups was comparable to controls on days 1, 17 and 21.

In a developmental rat study, MITC was given by gavage at 0, 3, 10 or 30 mg/kg bw/d. At the end of the treatment, several dams at top dose had sticky and/or moist fur in the area of the snout before, and at the same location reddish but mainly dry fur. Body weight and body weight gain were significantly reduced at top dose and marginally at intermediate dose. Water consumption was increased in individual dams at 10 and 30 mg/kg bw/d. Reproduction data were not significantly affected in the groups. The number of foetuses weighing < 75% of the mean fetal weight/litter was increased at top dose. Placenta weight was significantly lowered at top dose. Sex distribution was comparable in the different test groups.

One anomaly (anophthalmia) was detected in one foetus/one litter at 10 mg/kg bw/d. No retardations were seen in any group. There were no statistically significant differences between the treated groups and the controls with regard to anomalies, variations and/or retardations.

In rabbits, MITC was given by gavage at 1, 3 or 10 mg/kg bw/d. No test article related signs or symptoms were observed in any female of the different groups. At top dose, a slightly higher reduction of mean body weight was considered to be compound-related. Food consumption was reduced at top dose from day 6-11. No effects were seen on the mean number of implantations, pups or embryonic deaths. Body weight of fetuses were not affected. Sex ratio was similar in all groups. Investigations of the crania and body cavity and skeletal of the fetuses did not show compound-related effects.

Table B.6.10.1-11: Summary of reproductive toxicity and teratogenicity of MITC

Type of test Test species	Test substance purity	Results			References
		NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Symptoms	
2-generation rat study, drinking water at nominal concentration of 2, 10 or 50 ppm	Batch n° 340178; purity?	NOAEL parental toxicity= 10 ppm =0.76-1.01 mg/kg bw/d) NOAEL reproduction toxicity > 50 ppm =3.6 mg/kg bw/d NOAEL offspring toxicity >50 ppm (3.6 mg/kg bw/d)	50 ppm (3.6 mg/kg bw/d) - -	↓ body weight gain - -	Barker (1987), Barker (1986) and Jackson (1988)
Developmental rat study, gavage at 0, 3, 10 or 30 mg/kg bw/d day 6-15 gestation	Batch n° ZH 6205 MK; 96.9%	NOAEL maternal toxicity= 3 mg/kg bw/d NOAEL developmental toxicity=10 mg/kg bw/d	30 mg/kg bw/d 10 mg/kg bw/d	↓ body weight gain, ↑ water consumption ↓ fetal weight	Hellwig and Hildebrand, 1987
Developmental rabbit study, gavage 0, 1, 3 or 10 mg/kg bw/d days 6- 18	Batch n° ZNT 85/231-2; 98% w/w)	NOAEL parental toxicity= 3mg/kg bw/d NOAEL developmental toxicity=10 mg/kg bw/d	10 mg/kg bw/d -	↓body weight and body weight gain no effects	Becker et al, 1986

Summary of neurotoxicity studies

An acute neurotoxicity study was performed with metam sodium administered to rats at 50, 750 or 1500 mg/kg bw by gavage. In this study, a NOAEL systemic toxicity < 50 mg/kg bw/d was proposed and no neurotoxicity was seen in this study. NOAEL acute neurotoxicity > 1500 mg/kg bw/d. This study was conducted at very high dose levels and it is difficult to differentiate between direct and indirect effects on the nervous system. In any case, if metam should be considered as a neurotoxic substance, the effects reported in this study are observed at doses that are above the NOAEL for other toxic effects. Therefore, RMS concludes that the effects on the nervous system seen in this study are secondary to systemic toxicity.

In a repeat dose study, where metam sodium was administered in drinking water at doses going to 0.2 mg/ml (15-18 mg/kg bw/d), there were no signs of neurotoxicity.

Toxicity studies on metabolites

In soil, an average of 92% of metam sodium decomposes to MITC within a few hours and depending on soil moisture and soil decomposition, substantial amounts of MITC may remain in the soil for 12 days or more, in spite of the volatility of this compound. Photolysis of metam sodium in water and photolysis of MITC in air have been reported. Products in water include MITC and 1, 3-dimethylthiourea. Product in air includes methyl

isocyanate (MIC) methyl isocyanide, sulfur dioxide, hydrogen sulfide, carbonyl sulfide, N-methylformamide and methylamine. Methylisocyanate is more toxic than MITC and it as well as some of these other compounds may contribute to some of the toxic effects of metam sodium.

Thus, while metam sodium is itself non-volatile, its application results in significant production and emission of air contaminants. The half-life of MITC in air was reported as 29 to 39 hours in natural sunlight.

Therefore, following agricultural metam-sodium applications, inhalation exposure is essentially related to MITC, but might also include other degradation products such as CS₂, H₂S and MIC. There is uncertainty about how these breakdown products interact to produce the overall potential toxicity deriving from the use of metam-sodium, but MITC, MIC and H₂S have all been associated with ocular and respiratory irritation. While there are no data to address mixed exposure effects, additive or synergistic effects of MITC, MIC and H₂S in respiratory and ocular tissues are plausible.

A quite complete dossier related to **MITC** is included in the DAR. For MIC, the company did not provide studies. Therefore, RMS used data reported in the open literature. ECB (Ispra) reported a rationale for classification of methylisocyanate as T; R24/R25 for dermal and oral toxicity, T⁺, R26 for inhalation and respiratory tract irritation (R37). MIC is a skin irritant (Xi, R38) and an eye irritant (R41). MIC is highly toxic for humans and an accidental release in Bhopal (India) caused thousands of deaths by acute respiratory failure. Respiratory toxicity was also seen in animal studies. MIC was classified by ECB as T⁺; R26, T; R24/25, Xn; R42/43, Xi; R37/38/41. H₂S is a respiratory tract irritant and symptoms commonly reported after accidental human exposure include dyspnea, sore throat, coughing, chest pain and signs of pulmonary obstruction. Acute human exposure to CS₂ by inhalation leads to local irritation, pharyngitis and CNS toxicity. Methylamine is known for its irritating properties to eyes; nose and throat upon brief exposure, and severe exposure may lead to pulmonary edema.

COS toxicity was investigated in laboratory animals and CNS lesions were reported.

The company addressed the possible presence of MITC, MIC, CS₂ and methylamine under field conditions at toxicologically meaningful concentrations after metam sodium applications. As reported under point B.6.15.2 in the study of Links, 2006, levels of CS₂, methylamine, and MIC at day 0-4, after application of metam sodium at realistic application conditions, were <0.4 mg/m³, 0.027 mg/m³, and <0.004 mg/m³, respectively. From this study, it was concluded that the potential exposure of the operator to MIC and the stationary measurements to MIC inside the tractor were both below the limit of detection. MIC, CS₂ and methylamine were not detected around the treated field. At detection limit, the upper bound estimate for results of MIC for all field samples would be 4-µg/m³. For CS₂ this would be 400-µg/m³ and for methylamine, this would be 27-µg/m³. Air concentrations of MITC ranged from the limit of detection (0.042µg/m³) to a maximum concentration of 36-µg/m³ with a GM of 0.9 µg/m³ (n=96). The 95th percentile was 13.2 µg/m³. MITC concentrations in the tractor cabin were at the level of 0.070-0.076 mg/m³, during application reaching 0.107 mg/m³ outside the tractor. These results show that under realistic application conditions of metam sodium, while MIC, CS₂ and methylamine were below the limit of detection, MITC was identified at significant levels, confirming the need to assess operator exposure to MITC but not to MIC or other metabolites. The toxicity of MITC was investigated by the company and a full dossier is included and reported in parallel to metam sodium.

Medical data:

The signs of toxicity are likely to be lachrimation, blepharospasm, conjunctivitis and chemical burns after contact with eyes, primary irritation, dermatitis, eczema, chemical burns after contact with skin, nausea, vomiting, burns, gastroenteritis, sleepiness, cardiac and respiratory disorders, shock, renal lesions after ingestion, and primary mucosal and upper respiratory tract irritation, throat pain, cough hoarseness after inhalation.

B.6.10.2 Acceptable daily intake (ADI)

Metam sodium/MITC is phytotoxic and damage to plants is noticeable rapidly making the plant/commodities not suitable for use. In order to avoid the risk of phytotoxicity, soil fumigation occurs usually 3-4 weeks prior to planting. Labels require a period of 3 weeks after metam sodium application to allow for dissipation of phytotoxic MITC before planting a crop.

Metam sodium is not stable when diluted and breaks down rapidly into MITC, the half-life of which in soil is short, ranging from several hours to seven days.

Metam sodium has been tested in rats, mice, dogs and rabbits by oral route or by inhalation. Depending on the species, target organ is respiratory tract, urinary bladder or liver.

After long-term exposure (104-weeks) to metam sodium in drinking water, the lowest NOAEL was reported in the rat at 1.5 mg/kg bw/d. In mice, a quite similar result was reported (NOAEL = 1.9 mg/kg bw/d).

However, a short-term drinking water study showed lower NOAELs in dogs, and in a 1-year dog study a NOAEL was proposed at 0.1 mg/kg bw/d.

Therefore, RMS considers that this dog study is the most appropriate for setting of an ADI for metam sodium. Applying a safety factor of 100 gives an ADI = 0.001 mg/kg bw/d.

ADI metam sodium = 0.001 mg/kg bw/d

During the Peer-Review, the proposed ADI of Metam was confirmed.

For MITC, the lowest NOAEL = 0.4 mg/kg bw/d was reported in the 90-day dog drinking water study. (NOAEL revised upwards during the Peer-Review)

Applying a safety factor of 100 gives an ADI = 0.004 mg/kg bw/d.

ADI MITC = 0.004 mg/kg bw/d

The company had proposed to use the long-term drinking water study in mice with a NOAEL = 1.6 mg/kg bw/d (0.89 mg/kg MITC equivalent). Applying a safety factor of 100 gives an ADI = 0.02 mg/kg bw/d (0.009 mg/kg bw/d MITC equivalent).

B.6.10.3 Acute reference dose (ARfD)

Developmental toxicity was reported for metam sodium. In each of the reported studies, single cases of rare anomalies were reported at maternal toxic doses and detected in both rat and rabbit developmental studies. At maternal toxic doses, increased post implantation losses were reported.

Initially, RMS proposed to set an ARfD for metam sodium at 0.05 mg/kg bw/d, based on the NOAEL for developmental toxicity in rats at 5 mg/kg bw/d.

During the Peer-Review, the proposed ARfD of Metam was revised upwards. The meeting agreed the ARfD was 0.1 mg/kg bw/day based on overall rat developmental study and supported by rabbit developmental study.

ARfD metam sodium = 0.1 mg/kg bw/d

The company made no proposal

For MITC, an ARfD was initially proposed on the developmental toxicity NOAEL = 10 mg/kg bw/day reported in the rat study where pups showed a reduced body weight at 30 mg/kg bw/day.

However, during the Peer-Review, an ARfD of 0.03 mg/kg bw/day based on the NOAEL for rat maternal toxicity (at 3 mg/kg b.w./d in both the rat and the rabbit developmental study) with AF=100 was agreed upon.

ARfD MITC = 0.03 mg/kg bw/d

B.6.10.4 Acceptable Operator Exposure Level (AOEL)

From the chemistry of metam sodium, occupational exposure is most likely to the volatile breakdown product, MITC, rather than to metam sodium, itself.

RMS proposes to set an AOEL systemic toxicity for metam sodium based on the 90-day drinking water rat study where a NOAEL = 0.5 mg/kg bw/d is proposed. A correction for oral absorption is not required.

During the Peer-Review, the proposed AOEL of Metam was revised downwards. The AOEL originally proposed was 0.005 mg/kg bw/day, based on the 90-day rat drinking water study, with an SF of 100. It was agreed that the AOEL should be based on the one-year dog capsule feeding study at the same value as the ADI (0.001 mg/kg bw/day).

AOEL metam sodium = 0.001 mg/kg bw/d

In the initial DAR, the setting of an AOEL for operator exposure assessment on an oral study by gavage or drinking water was considered inappropriate for MITC, which is volatile, is the ultimate active substance, and is more toxic than the parent compound.

Inhalation toxicity of MITC was investigated after exposure of rats in a 4-week MITC inhalation study giving a NOAEL = 5 mg/m³ corresponding to 1.35 mg/kg bw/d.

Therefore, an NOAEL for inhalation of MITC was initially proposed at 5 mg/m³ corresponding to 1.35 mg/kg bw/d.

While initially, the RMS proposed an AOEL of 0.0135 mg/kg bw for MITC, based on a rat 4 wk inhalation study, this proposal was criticised by some MS. It was noted that the overall database for MITC was based upon oral studies, although inhalation was the critical route of exposure. It was remarked that the 4 wk study on MITC was also supported by the 90 day study using Metam. In addition, it could be argued that 4 wks would reasonably cover the use pattern of the product (e.g. greenhouse application).

However, taking into account the limited database for inhalation (only 4 wk study, and therefore of limited duration), it was considered by the meeting to use the NOAEL from 90 day oral dog study, as this duration could be considered more relevant for operator exposure (especially when the work of fumigation would be outsourced to contractors). In addition, the dog was considered the most sensitive species.

On balance, during PRAPeR meeting, the experts agreed that the 90 day dog study on MITC should be used to set the AOEL, and it was considered that a 4-week would not be sufficient to cover the application window. This study was also used for the ADI. The agreed AOEL was 0.004 mg/kg bw/day for MITC

AOEL MITC = 0.004 mg/kg bw/d.

Proposals from the company:

AOEL dermal (metam sodium) = 0.63 mg/person/day based on the 2 year carcinogenicity study in mice.

AOEL inhalation MITC = 1.7 mg/person/day based on the rat inhalation toxicity study.

B.6.10.5 Maximum acceptable concentration in drinking water

The determination of a MAC value is not necessary, because according to Directive 91/414/EEC only the ADI and AOEL values have to be determined. In addition to this, the maximum admissible concentration of an active substance is 0.1 µg/L, as established by the Directive 89/778/EEC.

B.6.11 Acute toxicity including irritancy and skin sensitization of the preparations (Annex IIIA 7.1)

Metam sodium is a soil fumigant acting by decomposition to MITC and used as a soluble concentrate (SL). Metam sodium is only available as an aqueous solution containing about 510 g/L a.i. and the product is equivalent to the technical active substance as manufactured. Therefore, all acute toxicity studies were conducted with aqueous metam sodium (or potassium) solutions as manufactured and are described in Annex II, point B.6.2.

B.6.11.1 Acute oral toxicity (Annex IIIA 7.1.1)

Please refer to Annex II, point B.6.2.1

B.6.11.2 Acute percutaneous toxicity (Annex IIIA 7.1.2)

Please refer to Annex II, point B.6.2.2

B.6.11.3 Acute inhalation toxicity to rats (Annex IIIA 7.1.3)

Please refer to Annex II, point B.6.2.3

B.6.11.4 Skin irritation (Annex IIIA 7.1.4)

Please refer to Annex II, point B.6.2.4

B.6.11.5 Eye Irritation (Annex IIIA 7.1.5)

Please refer to Annex II, point B.6.2.5

B.6.11.6 Skin sensitization (Annex IIIA 7.16)

Please refer to Annex II, point B.6.2.6

B.6.11.7 Supplementary studies for combinations of plant protection products (tests as at points 7.1.1 to 7.1.6) (Annex IIIA 7.1.7)

As Metam-sodium is not recommended to be used with other plant protection products or adjuvant as a tank mixture this point is not relevant.

B.6.12 Dermal absorption (Annex IIIA 7.3)

Excretion studies following oral administration have shown that a significant proportion of the dose is eliminated as metabolites (MITC, CS₂, COS) in exhaled air, and consequently an assessment of dermal absorption requires collection of exhaled metabolites. Metam sodium is unstable under acidic conditions and the degradation products are likely to be identical to the exhaled metabolites. Any collection of exhaled metabolites should include, under normal housing conditions, collection of the degradation products. As a result, dermal absorption of metam sodium could be overestimated.

B.6.12.1 Dermal absorption, in vivo in the rat (Annex IIIA 7.3)**- Pilot dermal absorption study with ¹⁴C metam sodium in female rats (Stewart, 1991)**

A preliminary study has been conducted to design an appropriate experimental procedure for the trapping of non-absorbed volatiles and expired metabolites. A saddle has been designed to provide a non-occluded, enclosed application site above which a pre-determined adsorbent (activated charcoal) has been suspended in order to adsorb degradation products.

Findings:

Following a single dermal application of 10 mg/rat for 10 h to two rats, a mean of 85% of applied radioactivity was recovered within 72 h, principally in the charcoal adsorbent washes (20-56.2%), application site washings (61-15%) and urine (4.1%). Radioactivity was only detected in the expired air trap designed to collect MITC (0.7%), with a maximum between 10 and 24 h after removal of saddle and CO₂ (1.9%). Radioactivity in faeces, carcasses, cage washings, air traps washings and traps were below the limit of detection throughout the study (table B.6.12.1-1).

Table B.6.12.1-1: pilot dermal absorption study with metam sodium (10 mg/rat).

Endpoints	Recovery of radioactivity (%) of individual results of 2 rats
Urine	
0-4 h	0.23-0.75
4-10 h	1.26-2.15
10-24h	1.72-1.13
24-48h	0.46-0.27
48-72 h	0.11-0.11
Total	3.78-4.41
Feces	
0-4 h	NS
4-10 h	NS
10-24h	0.11-0.05
24-48h	0.11-0.05
48-72 h	0.06-0.05
Total	0.28-0.15
Cage washes 0-72 h	0.4-0.75
Carcass digest	0.23-0.48
Application site	0.17-0.23
Blood	0.00
Air trap 1 (MITC)	0.94-0.37
Air trap 2 (CO ₂)	2.47-1.37
Air trap 3 (COS/CS ₂)	0.00
Adsorbent washes	19.94-56.20
Application site washes	60.83-14.87
Total recovered dose	89.71-80.22 % of applied dose

Conclusion:

Metam-sodium is not readily absorbed from the skin during the ten hour exposure period and <8.2% of the applied radioactivity was recovered in urine, faeces, expired air traps, cage wash and carcass.

GLP status: yes (no attest of competent authority)

Test guidelines: experimental protocol in compliance with OECD test guideline 427 (2004).

Material and methods: Non-radiolabelled Metam (batch n° CP 2891) and radiolabelled ¹⁴C metam (batch n° BAS 00500N (390/2) > 99percentage purity and mean specific radioactivity = 72.59 µCi/mg) were used. Radiolabelled Metam was purified by TLC. 2 Crl:CD (SD) BR female rats were exposed to metam at 10 mg/rat dissolved in 0.02M phosphate buffer corresponding to 50µl/rat (= 200 g/L), pH 8 (stability was acceptable) for a 10 h application.

24 h prior to dosing, an area of dorsal-lumbar skin was shaved and saddle was fixed to the middle of the back of each rat. 24 h after the saddle application, rats were anesthetized under halothane, metam was applied over the tested area, animals are placed in a metabolism cage and excreta, and expired air was collected over a 72 h post dose interval. At termination of experimental phase, the rats were anesthetized, saddle contents removed, and application site was washed with a detergent. Expired air was scrubbed in 3 traps, one for MITC, one for CO₂ and one for COS and CS₂, the efficiency of the traps was found adequate. The radioactivity present in urine, faeces, air traps, cage washings, cage debris, application site washings, cotton wool swabs(group D), activated charcoal, activated charcoal washings and sinter (including gauze, foam and elastic bands) washings were determined.

The study is accepted.

- Main study: *in vivo* percutaneous absorption study of metam sodium in the rat (Stewart, 1992)

Findings:

Due to the instability of metam sodium when formulated as a very dilute aqueous solution (0.2 mg/ml = 0.01 mg/animal) animals were not dosed at 0.01 mg.

No overt pharmacological or toxicological signs were observed during the course of the study.

Following nominal doses of 0.1, 1 and 10 mg/animal, overall mean recoveries of radioactivity were 95.7%, 90.5% and 83.5%, respectively.

The total dose recovered in washings was in the range of 71-93% for low dose, 66-82% for intermediate dose and 64-80% for the high dose level.

The dose retained in the skin increased with time up to 24 h for the low and intermediate dose, but decreased as a function of time for the high dose.

Excreted dose increased at low and intermediate dose as a function of time reaching a maximum at 24 h and decreasing at 72 h while at high dose a maximum of excretion was noted at 2 h post application.

A substantial proportion of each dose volatilized from the skin surface during each experiment. This volatility could also account for the incomplete recoveries. Some of the radioactivity recovered from the expired air traps, particularly air trap 2, may have originated because of losses directly from the skin and might, therefore, not all be part of the absorbed dose. This conclusion is drawn since some results for expired air trap 2 are inconsistent with the majority of results for the other groups. When excretion was monitored over 72 h, following a 10 h exposure period, the majority of the absorbed dose was eliminated within the 24 h period after dosing.

Substantial quantities of non-absorbed dose were recovered from the charcoal present in the saddle resulting from the high volatility of metam and its degradation products (table B.6.12.1-2).

Following applications at nominally 1 and 10 mg/animal, concentrations of radioactivity in blood and plasma peaked at 1-hour post dose, thereafter levels fell (Table B.6.12.1-3).

Although the company proposed not to take into account the dose retained in skin, RMS considers that the excretion of radioactivity has not ended within the 72 h sampling time, therefore, the amount located in the skin should be considered as being absorbed. This gives a dermal absorption of 16.5% after low dose, 8.8% after an intermediate dose and 4.6% after a high dose.

Table B.6.12.1-2: *in vivo* dermal absorption study with metam sodium.

Endpoints/ Time	% of administered dose				
	1 h	2 h	10 h	24 h	72 h
0.1 mg (0.0086mg/cm² = 2 g/L)					
Charcoal + washings	13.69+50.5 = 64.17	29.20+44.9 = 74.08	35.01+48.6 = 83.57	26.42+36.0 = 62.42	32.15+52.4 = 85.6
Sinter washings	9.457	2.190	0.033	1.377	0.519
Application site washings	19.18	11.34	8.135	7.491	6.840
Total unabsorbed dose : %	92.8	87.6	91.7	71.3	92.9
Treated skin	2.663	3.575	7.741	6.423	2.794
Untreated skin	0.075	ND	ND	ND	ND
Carcass	ND	ND	ND	ND	ND
Blood	0.016	0.014	0.023	0.036	0.0157
Urine	ND	0.018	0.451	1.002	1.049
Feces	ND	ND	ND		
Cage washings	ND	ND	0.081	0.015	0.100
Air traps 1/2/3	0.008/1.783 /ND	ND/2.161/ND	ND/1.824/ND	0.180/6.849/ND	0.041/1.235/ND
Excreted dose:	1.791	2.179	2.356	8.04	2.42
Total absorbed dose: %					
- skin	1.807	2.193	2.379	8.082	2.440
+ skin	4.47	5.76	10.12	16.50	5.23
Total recovery %	97.33	93.37	101.8	87.79	98.17
1 mg (0.0862mg/cm² = 20 g/L)					
Charcoal + washings	1.345+7.37 = 8.721	24.77+0.013 = 24.78	38.46+0.143 = 48.6	40.06+0.177 = 40.23	51.53+7.143 = 58.67
Sinter washings	1.025	0.053	0.143	0.177	7.143
Application site washings	72.97	41.35	26.68	29.75	11.40
Total unabsorbed dose:	82.7	66.1	75.4	70.15	77.2
Carcass	0.144	0.266	ND	0.815	ND
Blood	0.032	0.038	0.011	0.0072	0.0095
Treated skin	3.623	6.731	4.310	4.107	1.633
Untreated skin	ND	0.046	ND	0.130	ND
Urine	0.011	0.044	0.749	1.321	0.697
Feces	ND	ND	0.026	0.701	0.016
Cage washings	0.022	ND	0.087	0.161	0.129
Air traps 1/2/3	ND/1.707/ND	0.016/2.761/ND	0.029/2.424/0.37	0.097/1.623/0.005	0.160/2.165/ND
Excreted dose	1.74	2.82	3.68	3.90	3.16
Total absorbed dose:					
- skin	1.915	3.124	3.694	4.729	3.176
+ skin	5.53	9.85	8.0	8.83	4.8
Total recovery	88.22	88.08	98.15	85.33	92.45
10 mg (0.862mg/cm² = 200 g/L)					
Charcoal + washings	0.77+5.63 = 6.403	0.78+44.9 = 45.68	0.74+19.36 = 20.10	0.32+21.92 = 22.24	0.24+20.65 = 20.88

Endpoints/ Time	% of administered dose				
	1 h	2 h	10 h	24 h	72 h
Sinter washings	0.033	1.818	0.028	0.077	0.004
Application site washings	57.67	31.83	60.68	57.77	58.19
Total unabsorbed dose:	64.11	79.34	80.82	80.10	79.07
Carcass	ND	0.432	0.342	0.541	1.217
Blood	0.023	0.0207	0.0112	0.0102	0.004
Treated skin	14.88	1.683	1.294	2.391	0.700
Untreated skin	ND	0.071	0.016	0.081	ND
Urine	ND	0.079	0.489	0.753	0.942
Feces	ND	0.044	0.021	0.459	0.109
Cage washings	0.064	0.062	0.106	0.157	0.145
Air traps 1/2/3	ND/0.324/ND	0.245/4.364/ND	0.072/0.483/0.001	0.432/0.193/ND	0.353/0.748/ND
Excreted dose	0.388	4.79	1.17	1.69	2.29
Total absorbed dose:					
- skin	0.411	5.246	1.525	2.245	3.517
+ skin	15.29	6.92	2.82	4.63	4.2
Total recovery	79.38	86.35	83.4	84.80	83.29

ND: not detected; NA not applicable

Table B.6.12.1-3: concentration of radioactivity in whole blood and plasma following a single dermal application of metam of an aqueous formulation.

Endpoints/ Time	µg equivalents metam sodium/g tissue				
	1 h	2 h	10 h	24 h	72 h
Whole blood					
0.1 mg	ND	ND	ND	ND	ND
1 mg	0.038	0.044	0.014	0.012	ND
10 mg	0.341	0.280	0.187	0.125	ND
Plasma					
0.1 mg	ND	ND	ND	ND	ND
1 mg	NS	0.008	ND	NS	ND
10 mg	NS	NS	NS	NS	NS

Conclusion:

Following a single dermal application of metam sodium at dose levels of 0.1, 1 and 10 mg/animal, the extent of absorption was low. Total absorbed dose at sampling time of 24 h represents 16.5 %, 8.8 and 4.6% of the low, middle and high dose, respectively.

According to the company, the dermal absorption *in vivo* after application of 0.1, 1.0 and 10 mg Metam-sodium/rat (corresponding to 8.62, 86.2 and 862 µg/cm² applied on a total area of 11.6cm²/male rat) was in the range of 2.4 – 3.5 % after 72 h with a skin wash after 10 h, respectively. Without skin wash dermal absorption after an exposure time of 1, 2, 10 and 24 h was comparable at the different observation time points and determined to be in the range of 1.5 – 5.1 %, respectively.

GLP status: yes (no attest of competent authority)

Test guidelines: experimental protocol in compliance with OECD test guideline 427 (2004).

Material and methods: Four Crl: CD (SD) BR male rats/group were exposed to ¹⁴C Metam sodium labeled on the same position as that used for oral absorption study (batch n° 342-01 and batch n° Y06930/003/005; specific radioactivity for both batches was 64.49 µCi/mg) and non-radiolabelled metam (batch n° 42/137-1, 99.4%). Test article was prepared as a solution in HPLC grade water to provide a nominal dose volume of 0.05 ml/animal. For dose groups C and D, each dose was administered within 4 hours of the specific radioactivity and homogeneity checks. For group B, the test article was dissolved in 10% of the required volume of formulation vehicle. Stability of the remaining aqueous formulations was established.

Metam sodium was applied on a skin area of 11.6cm²/male rat, at a nominal dose of 0.1 mg (group B; 8.62 µg/cm²), 1 mg (group C; 86.2 µg/cm²) and 10mg/animal (group D corresponding to a solution at 200 g/L; 862 µg/cm²) corresponding to a radioactive dose of 7 µCi (B)

and 20 μCi (C and D). Group A was planned to receive 0.2 mg/l but the solution was unstable and the animals were not dosed. The dose formulation was spread evenly over the skin within the area enclosed by the glass saddle containing an activated charcoal filter. The charcoal and sinters were secured by a foam disc, cotton gauze and an elastic band. Sham dosing was performed at regular intervals, e.g. sham, animal, sham, animal etc for all animals in dose groups B and C: sham doses (0.05 ml) were dispensed into volumetric flasks (routinely 10 ml) and 2-ethoxyethanol was subsequently added. Following serial dilutions with 2-ethoxyethanol, radioactivity in each sham dose was determined by LSC. Following application of metam sodium, rats were placed in individual all-glass metabolism cages and expired air was drawn through 3 traps in series: trap 1: 2-ethoxyethanol to trap MITC, trap 2, ethanolamine- 2-ethoxyethanol (1/3) to trap CO₂ and trap 3 containing Viles reagent to trap CS₂. This trap was maintained on water ice. Four animals were killed following a 1, 2, 10 and 24 h exposure after administration. Four additional rats/group had the treated area washed 10 h after administration and excretion monitored over a total of 72 h.

An interim backwash at 10 h post dose was carried out on animals to be killed at 72 h. Wash and extraction of material was done with 2-ethoxyethanol and 3-x methanol. The treated skin area was swabbed thoroughly with detergent and water before rinsed and dried with cotton wool swabs.

The study is accepted.

B.6.12.2 Comparative dermal absorption, *in vitro* using rat and human skin (Annex IIIA 7.3)

- *in vitro* absorption through rat and human skin (Clowes, 1993)

Findings: the study was performed with a solution at 235 mg/ml corresponding to 0.940mg/cm² and at 23.5 mg/ml (0.094 mg/cm²) in water. Full thickness skin from human and rat was used.

At 10 h after dermal application, the skins were washed. The absorption was found to be dose and time dependent through both human and rat skin.

Definition of absorbed material: in this study, the compound is defined to be absorbed once it has diffused through the skin membrane and has entered the receptor chamber of the diffusion cell.

After high dose exposure, at 10 h, 21.3% of the dose was absorbed through rat skin and 2.19% from human skin. For the low dose exposure, 19.4% of the dose was absorbed from rat skin and 12.2% from human skin. At 10 h after application, a greater proportion of the applied dose was washed off the skin than was absorbed. The percentage of the dose remaining in the skin increased with decreasing dose.

As the dose in receptor chamber increased with time RMS considers that compound, which is located in the skin could be absorbed.

Already Peer Reviewed, EFSA 2008

Open point: 2.16 RMS to provide an addendum with revised data from the dermal absorption study [See reporting table 2(49)]

Open point: 2.17 Dermal absorption values to be agreed on in a meeting of experts

See reporting table 2(50)

Please refer to revised table B.6.12.2-1 (changes highlighted)

Table B.6.12.2-1: *in-vitro* comparative dermal absorption study parameters at 10 h.

Endpoints /time	Absorbed dose in $\mu\text{g}/\text{cm}^2$ (%)			
	Rat		Human	
Applied dose	940 $\mu\text{g}/\text{cm}^2$	94 $\mu\text{g}/\text{cm}^2$	940 $\mu\text{g}/\text{cm}^2$	94 $\mu\text{g}/\text{cm}^2$
Receptor chamber				
0.5 h	41.0 (4.4)	7.72 (8.2)	11.8 (1.2)	8.10 (8.5)
1 h	69.9 (7.4)	10.3 (10.9)	12.7 (1.34)	8.50 (9.01)
2 h	97.9 (10.4)	12.3 (13)	14.3 (1.5)	9.24 (9.8)
4 h	136 (14.4)	14.4 (15.2)	15.6 (1.65)	10.1 (10.7)
6 h	167 (17.7)	16.3 (17.2)	16.7 (1.77)	10.6 (11.2)
10 h	200 (21.3)	18.2 (19.4)	20.6 (2.19)	11.5 (12.2)
Wash at 10 h	590 (62.8)	47.2 (50.2)	712 (75.7)	52.0 (55.3)
Skin at 10 h	34.4 (3.66)	7.74 (8.23)	36.3 (3.86)	6.49 (6.91)
Donor at 10 h	80.6 (8.58)	5.91 (6.28)	199 (21.2)	18.3 (19.5)
Filter at 10 h	53.1 (5.65)	9.69 (10.3)	35.3 (3.75)	7.28 (7.74)
% total recovered	102	94.4	907	102
Absorbed dose (%) *	203.66 (24.96)	26.4 (27.63)	24.5 (6.05)	18.41 (19.11)

*Absorbed dose= dose in receptor chamber + dose retained in skin

Revised absorption values: already Peer-Reviewed, EFSA 2008

Conclusion:

In this *in-vitro* study, the application of a high dose of Metam-sodium (235 mg/mL or 940 $\mu\text{g}/\text{cm}^2$) resulted in a dermal absorption of 24.96 % and 6.05 % of the applied dose in rat skin and human skin while at the low dose (23.5 mg/mL or 94.0 $\mu\text{g}/\text{cm}^2$) dermal absorption in rat and human skin amounted to 27.6 % and 19.11 % of the applied dose, respectively. These results take into account the residual radioactivity remaining in the skin.

Dermal absorption of metam through rat skin is 4.1 times (24.96÷6.05) higher than that observed with human skin when a high dose is applied and 1.4 (27.6÷19.11) times higher than with human skin when a low dose is applied.

The *in-vivo* dermal absorption in rats is adjusted in light of the relative absorption through rat and human skin. The results from the *in vivo* rat study showed a dermal absorption of 16.5% after low dose, 8.8% after an intermediate dose and of 4.6% after a high dose.

$$\text{In-vivo human absorption} = \text{in-vivo animal absorption} \times \frac{\text{in-vitro human absorption}}{\text{in-vitro animal absorption}}$$

This corresponds to a value of 12% (16.5÷1.4) for a low dose and 1.1% (4.6%÷4.1) for a high dose.

However, for the calculation of the dermal absorption in human *in-vivo* the dermal penetration results for the concentrate are considered only since Metam-sodium is used as marketed, i.e. 510 g/L SC formulation.

Consequently, the results obtained in the *in-vitro* dermal penetration studies performed with human skin represent a worst case since they were performed with a less concentrated formulation (235 mg/mL) which in general penetrate skin at a higher rate as the more concentrated formulation.

The company proposed a value of 0.35%.

GLP status: yes (no attest of competent authority)

Test guidelines: experimental protocol not fully in compliance with OECD test guideline 428 (2004).

Deviation from official protocol: application time of 10 h instead of and temperature 2°C too low. Skin thickness not reported.

Material and methods:

Unlabelled metam (CTL Y06930/009, BASF lot n° 42/137-1, 98.5%) and radio labeled metam (^{14}C , CTL Y06930/003; 75.3% purity; 0.788 MBq/mg) was diluted in water in order to have a solution at 235 mg/ml (940 $\mu\text{g}/\text{cm}^2$) and at 23.5 mg/ml.

Skin of male Wistar rats was prepared and any subcutaneous fat was removed and freeze-dried. The skins were used within 7 days of preparation. Human abdominal skin was obtained post mortem and subcutaneous fat and muscle were removed. The skin was stored in a freezer until required but within 7 days of preparation.

An area of 2.52 cm^2 of skin was available for absorption and experiments were conducted in a water bath at 30°C. Receptor solutions were stirred for the duration of experiment.

Barrier integrity of skin samples was assessed by measuring their permeability to tritiated water.

Diffusion cells using skin from at least three cadavers were set up for each 10 h exposure time. ^{14}C metam was applied to the skin at a rate of four $\mu\text{l}/\text{cm}^2$ (10.2 $\mu\text{l}/\text{cell}$) to give dose rates of 940 $\mu\text{g}/\text{cm}^2$ and 94 $\mu\text{g}/\text{cm}^2$. The aqueous applications were spread over the skin surface and the donor chambers covered with two porous carbon filter discs to trap any evaporating dose. The receptor fluid was water. The glass rods were placed in individual capped pots to which 50% ethanol/water (5 ml) was added. Apart from the presence of carbon filters, the skin was left un-occluded for the duration of the experiment. At 10 h, the skin exposure was terminated by removal of the donor chamber and the receptor solution. The carbon filters were placed in capped pots containing 50% ethanol/water for at least 0.5 h. residual donor material was removed from the exposed surface of the skin by rinsing with 3% Teepol in water. Skin was dissolved before counting. Samples were taken from the receptor solution at 0.5, 1, 2, 4, 6 and 10 h and original volume was restored. The study is accepted.

B.6.13 Toxicological data on non active substances (Annex IIIA 7.4 and point 4 of the introduction)

Please, refer to doc J.

B.6.14 Summary of toxicity of the formulation metam sodium

Metam sodium is only available as an aqueous solution containing about 510 g/L a.i. and the product is equivalent to the technical active substance as manufactured. Therefore, all acute toxicity studies were conducted with aqueous metam sodium (or potassium) solutions as manufactured and are described in Annex II, point B.6.2.

B.6.15 Exposure data (Annex IIIA 7.2)

Note for the revised DAR, prepared for the re-submission of the a.s. in July 2010.

The field and greenhouse trials were revisited, and operator, worker and bystander exposure to MITC re-assessed, based upon the conclusions of the EFSA and LoEP subsequent to the Peer-Review in 2008. Input parameters were harmonised (70 kg b.w. for operators, and 60 kg b.w. for workers, bystanders and residents). The working duration was 7h/d for operators and 8h/day for workers. Bystanders were considered to be present in the neighbourhood of treated fields or greenhouses during one hour. Resident exposure was extrapolated for a 24h stay at more remote distances from the treated greenhouse (new trial). The AOEL of MITC was 0.004 mg/kg b.w./d. The exposure values based upon the agreed input parameters were compared with the revised AOEL, and the conclusions were amended accordingly. For the sake of transparency, the agreed values were highlighted in yellow in the original text.

One new greenhouse study was added to the data package. Amendements and new data were highlighted in yellow. The new trial (reference IIIA 7.2.3 /03) was evaluated under **B.6.15.2.4** (*Operator exposure towards MITC after application of Metam sodium in the greenhouse*), "Second greenhouse trial (drip irrigation), evaluated during re-submission in July 2010". All results (those agreed upon and concluded in the EFSA Report, and the new ones) are integrated in the final conclusions under B.6.15.7 "Operator, worker and bystander exposure to MITC after application of Metam".

Metam-sodium is a soil fumigant which is active against nematodes, soil-borne diseases, weed seeds, seedlings and soil pests. Metam-sodium is acting by decomposition to Methylisothiocyanate (MITC) and is used as a soluble concentrate (SL) containing 510 g/L (42.1 % w/w) Metam-sodium.

Metam-sodium is used to prepare soils for planting of crops like potatoes, cabbage, carrots, strawberries, melons, grapevine and orchards (see table B.6.15-1).

Application of Metam-sodium is carried out by a soil injection technique using tractor-mounted equipment as well as by a drip irrigation technique the latter of which does not require the presence of an operator except for coupling the drum to the drip irrigation installation. In addition, Metam-sodium is also applied for soil fumigation in greenhouses.

Metam-sodium is applied at a rate of up to 1200 L/ha corresponding to 612 kg Metam-sodium/ha and the product will be marketed in 60, 200 and 1,000 L containers or drums.

For the application of Metam-sodium for soil fumigation purposes the following application techniques are considered in this document:

- 1) Soil injection of the undiluted formulation followed by incorporation by rotavation and soil surface sealing with a roller (carrots, lamb's lettuce, potato, tomato) in field and in greenhouses.
- 2) Drip irrigation under plastic film on the planting row (cucumber, eggplant, pepper, tomato, strawberry) or on the planting row in glasshouse).

In order to clarify the different uses of Metam, the EU-GAP table is represented in table B.6.15-0. Two remarks are made:

(i) the term "no dilution" was used in the sense that Metam is not diluted *prior* to application, contrary to what is common practice for most plant protection products. Metam is not to be loaded and mixed in a tank, so there is no "spray solution". In the case of drip irrigation with Metam the dilution with water takes place during the actual application, not in a tank but in the tubes of the irrigation system with instantaneous application.

(ii) It was of note that there was no exposure study in order to support the soil injection at 40 cm at the site of the de-rooted vine (0.2L/m²) in grapes. However, although the intended use in grapes is at the higher dose of 1020 kg a.s./ha, only an occasional treatment would be necessary (isolated cases of few trees in a row of vines), and the spot treatment at the foot of the de-rooted vine would most likely lead to a lower exposure of the operator than the treatment of a full field. The application rate on full field basis will be considerably lower than 1020 kg a.s./ha. Furthermore, the time between disinfection and the harvest of new grapes is considerably longer than in vegetable growing, therefore residue trials in this crop were considered less relevant. Taking this into account, the RMS considers this use in the EU-GAP table to be acceptable and covered by the risk assessments performed

for the other intended uses. Refinements may however be considered at Member State level, if deemed necessary.

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Table B.6.15-0: Detail of intended uses (updated GAP information)

Crop and/or situation (a)	Member state or country	Product name	F G or I (b)	Pests or Group of pests controlled (c)	Formulation		Application				Application rate per treatment			PHI (days) (l)	Remarks (m)
					Type (d-f)	Conc. of a.s. (i)	method kind (f-h)	growth stage & season (j)	number min max (k)	interval between applications (min)	kg a.s./hl min max	water l/ha min max	kg a.s./ha min max		
Carrot	DE, BE, CY, ES, F, MT, EL, PL, HU, EI, IT, NL, P, UK		F	Soil fumigant	SL	510 g/l	Soil injection + incorporation at 25 cm by rotavation	April-June	1	n.a.	n.a.	No dilution	408	n.a.	
Carrot	DE, BE, CY, ES, F, MT, EL, PL, HU, EI, IT, NL, P, UK		F	Soil fumigant	SL	510 g/l	Soil injection + incorporation at 25 cm by rotavation	Oct-Dec	1	n.a.	n.a.	No dilution	408	n.a.	
Corn salad (=lamb's lettuce)	F, ES, BE, GE, IT		F	Soil fumigant	SL	510 g/l	Soil injection + incorporation at 15 cm by rotavation	April-Aug	1	n.a.	n.a.	No dilution	306	n.a.	
Cucumber	DE, BE, CY, ES, F, MT, EL, PL, HU, EI, IT, NL, P, UK		G	Soil fumigant	SL	510 g/l	Drip-irrigation under a plastic film on the planting row	June-Aug	1	n.a.	n.a.	45000-180000	459	n.a.	Diluted with water directly in the drip irrigation lines at concentration 0.5-2.0 %

Crop and/or situation (a)	Member state or country	Product name	F or I (b)	Pests or Group of pests controlled (c)	Formulation		Application				Application rate per treatment			PHI (days) (l)	Remarks (m)
					Type (d-f)	Conc. of a.s. (i)	method kind (f-h)	growth stage & season (j)	number min max (k)	interval between applications (min)	kg a.s./hl min max	water l/ha min max	kg a.s./ha min max		
Eggplant / aubergine	CY, ES, F, MT, EL, IT, NL, P		G	Soil fumigant	SL	510 g/l	Drip-irrigation under a plastic film on the planting row	June-Aug	1	n.a.	n.a.	60000-240000	612	n.a.	Diluted with water directly in the drip irrigation lines at concentration 0.5-2.0 %
Pepper	BE, CY, ES, F, MT, EL, PL, HU, IT, NL, P, UK		G	Soil fumigant	SL	510 g/l	Drip-irrigation under a plastic film on the planting row	June-Aug	1	n.a.	n.a.	60000-240000	612	n.a.	Diluted with water directly in the drip irrigation lines at concentration 0.5-2.0 %
Potato	DE, BE, CY, ES, F, MT, EL, PL, HU, EI, IT, NL, P, UK		F	Soil fumigant	SL	510 g/l	Soil injection + incorporation at 25 cm by rotavation	Sept-Nov	1	n.a.	n.a.	No dilution	153	n.a.	
Strawberry	DE, BE, CY, ES, F, MT, EL, PL, HU, EI, IT, NL, P, UK		F	Soil fumigant	SL	510 g/l	Drip irrigation under a plastic film on the planting row	Jul-Oct	1	n.a.	n.a.	60000-240000	612	n.a.	Diluted with water directly in the drip irrigation lines at concentration 0.5-2.0 %

Crop and/ or situation (a)	Member state or country	Pr od uct na me	F G or I (b)	Pests or Group of pests controlled (c)	Formulation		Application				Application rate per treatment			PHI (days) (l)	Remarks (m)
					Type (d-f)	Conc. of a.s. (i)	method kind (f-h)	growth stage & season (j)	number min max (k)	interval between applications (min)	kg a.s./hl min max	water l/ha min max	kg a.s./ha min max		
Tomato	DE, BE, CY, ES, F, MT, EL, PL, HU, EI, IT, NL, P, UK		G	Soil fumigant	SL	510 g/l	Drip irrigation under a plastic film on the planting row	Feb-Aug	1	n.a.	n.a.	60000- 240000	612	n.a.	Diluted with water directly in the drip irrigation lines at concentra- tion 0.5- 2.0 %
Tomato	DE, BE, CY, ES, F, MT, EL, PL, HU, EI, IT, NL, P, UK		F	Soil fumigant	SL	510 g/l	Soil injection at 5 cm + coverage by 10 cm of soil	March	1	n.a.	n.a.	No dilution	612	n.a.	
Grape	F, IT, DE, ES, EL, HU, P		F	Soil fumigant	SL	510 g/l	Soil injection at 40 cm at the site of the de-rooted vine (0.2 l/m²)	Autumn. Before replantin g	1	n.a.	n.a.	No dilution	1.020	n.a.	

Remarks:

(a) For crops, the EU and Codex classification (both) should be used where relevant, the use situation should be described (e.g. fumigation of a structure)

(b) Outdoor or field use (F), glasshouse application (G) or indoor application (I)

(c) e.g. biting and suckling insects, soil born insects, foliar fungi, weeds

(d) e.g. wettable powder (WP), emulsifiable concentrate (EC), granule (GR)

(e) GCPF Codes - GIFAP Technical Monograph No 2, 1989

(f) All abbreviations used must be explained

(g) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench

(h) Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated

(i): g/kg or g/l

(j) Growth stage at last treatment (BBCH Monograph, Growth stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application

(k) The minimum and maximum number of application possible under practical conditions of use must be provided

(l) PHI - minimum pre-harvest interval

(m) Remarks may include: Extent of use/economic importance/restrictions

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Table B.6.15-1: Summary of use pattern, application rates and equipment

Crop treated	Application rate of metam-sodium		Field/ Greenhouse	Method
Carrot	800 L/ha	408 kg a.s./ha	Field	Soil injection + incorporation on 25cm depth by rotovation
Corn salad (lambs lettuce)	600 L/ha	306 kg a.s./ha	Field	Soil injection + incorporation on 15cm depth by rotovation
Tomato	1200 L/ha	612 kg a.s./ha	Field	Soil injection at 5 cm depth + coverage by 10 cm of soil
Potato	300 L/ha	153 kg a.s./ha	Field	Soil injection + incorporation on 25cm depth by rotovation
Strawberry	1200 L/ha	612 kg a.s./ha	Field	Drip-irrigation under a plastic film on the planting row
Cucumber	900 L/ha	459 kg a.s./ha	Greenhouse	Drip-irrigation of a 0.5-2% dilution on the planting row
Eggplant, pepper, tomato	1200 L/ha	612 kg a.s./ha	Greenhouse	Drip-irrigation of a 0.5-2% dilution on the planting row

For the estimation of operator exposure in soil fumigation applications, no generally accepted exposure assessment model is available. When soil fumigants such as Metam-sodium, which decomposes to the active principle Methylisothiocyanate (MITC), are considered, then exposure towards MITC needs to be taken into account.

The application of Metam-sodium by soil injection and drip irrigation is performed in the open field and in the greenhouse.

Operator exposure towards Metam sodium in greenhouses is considered different to the exposure of operators in the open field, because both the application mode and applied concentration will be different.

In open field, Metam will be applied by soil injection with the 50% SL (note: chemigation is not an acceptable application mode).

In tunnels or greenhouses, the drip-irrigation of Metam 50% S, diluted to a concentration of 0.5-2%, will be considered the only acceptable agricultural practice.

The application scenarios for Metam-sodium, which comprise soil injection and drip irrigation and associated exposure to operators during mixing/loading processes and during application differ profoundly from other application techniques (such as spraying by tractor-mounted equipment with hydraulic nozzles). Therefore, the conventional models for operator exposure were considered irrelevant. Automated direct transfer systems will be used for mixing/loading procedures resulting in negligible exposure. For the application of Metam-sodium by drip irrigation mixing/loading comprises only connecting and disconnecting of pumping systems to or from the storage containers. In addition Metam-sodium is rapidly transformed into MITC. The existent models provide no tools for the estimation of operator exposure to metam sodium which is a liquid giving rise to the gaseous and volatile metabolite MITC, formed quite rapidly when metam-sodium enters into contact with soil.

Exposure of operators towards MITC during application was measured in different independent operator exposure trials after soil injection application of Metam-sodium by tractor-mounted equipment, and after drip-irrigation in a greenhouse.

B.6.15.1 Estimation of operator exposure (Annex IIIA 7.2.1.1)

For the assessment of operator exposure towards MITC during soil injection application exposure measurements were performed in the South and in the North of the Netherlands, and in the under representative field conditions (i.e. application rate and application techniques).

The company provided further information on the proposed personal protective equipment and on the application technology:



the company recommend wearing face-sealing goggles, water proof gloves and coveralls during handling, as well as respiratory protection with vapour removing cartridges (TC-23C or TC-14 G, A2B2) and chemical resistant foot wear. As respiratory protective equipment the company promotes the use of the helmet which is more user-friendly than the mask as it facilitates breathing. During the treatment, a coverall, water proof gloves, chemical resistant footwear are obligatory. If a strong odour is detected, we also recommend the face goggles and respiratory protection.

Technologies of application : mechanised or drip-fumigation

Application by drip irrigation

Use is made of the existing irrigation network in greenhouses and/or under plastic sheeting. An automatic pumping system dilutes Metam-sodium aqueous solution with the desired quantity of water. The dilution factor is based on the application rate per ha of treated soil surface and the quantity of water normally needed to irrigate the soil to a certain depth (root area).

There is virtually no contact to the product: in the beginning of the application the operator (mixer/loader) has to dip a tube in the drum of –undiluted– metam-sodium solution. Then a pump is turned on, which is automatically diluting the metam and sending it to the irrigation tubes. At the end of the application, the operator removes the tube from the drum and puts it in a bucket of clear water. This way, the entire system is rinsed with water, which is necessary because of the phytotoxic nature of metam.

Mechanised application

Specialised equipment injects the metam-sodium aqueous solution (undiluted) directly into the soil. There is a trend towards using tractors equipped with pressurised cabins. The loading of the tank is done by pumping the product directly from the drum or container. There is virtually no contact with the product.

Modern machines are already equipped with a fixed pumping system and pumping tubes (attached to the machine). In the latest system for transferring to product, a 500 Litre drum is installed automatically on the machine (no heavy weight for the worker) and fitted with a dry coupling system (no leakage).

Conclusion

Metam (-sodium) is not applied by spraying but by special application techniques such as drip irrigation and mechanized soil incorporation. These techniques preclude exposure to metam-sodium via spray drift or spillage during manual emptying of containers into the spray tank. In combination with the recommended personal protective equipment, the use of metam-sodium according to the GAP is deemed to be safe for the operator/mixer/loader.

B.6.15.1.1 Estimation of operator exposure to Metam-sodium (Annex IIIA 7.2.1.1)

Not relevant, as no exposure to Metam-sodium (approved PRAPeR Peer Review 2008).

B.6.15.1.2 Estimation of operator exposure to MITC (Annex IIIA 7.2.1.1)

No model is available for the estimation of operator exposure to MITC after soil injection application of Metam-sodium.

For the assessment of operator exposure towards MITC during soil injection application exposure measurements were performed in the South and in the North of the Netherlands under representative field conditions (i.e. application rate and application techniques) the results of which are described below.

B.6.15.2 Measurement of operator exposure (Annex IIIA 7.2.1.2)

B.6.15.2.1 Measurement of operator exposure to metam sodium (Annex IIIA 7.2.1.2)

Not relevant, as no exposure to Metam-sodium (approved PRAPeR Peer Review 2008).

B.6.15.2.2 Measurement of operator exposure to MITC after field application of metam sodium (Annex IIIA 7.2.1.2)

To assess operator exposure towards MITC during application of metam, three operator exposure studies were performed in the Netherlands.

Metam-sodium was applied by soil injection using representative scenarios with respect to application techniques (cultivator injector, spade injector and Rumpstad shear injector), application rates and soil types.

Exposure measurements on MITC comprised personal measurements of the operator/tractor driver at the lapels, measurements in the tractor cabin and measurements outside the tractor cabin near the tractor driver. Only the inhalation route of exposure was taken into account for MITC exposure measurement. Dermal exposure towards MITC is considered negligible due to the availability of the substance in the gaseous phase only after decomposition of Metam-sodium. Furthermore, the MITC exposure results obtained in these exposure studies after application of Metam-sodium by a soil injection technique do represent a worst case since for drip irrigation application where no operator has to be present during application and thus, no to negligible exposure towards MITC is expected.

The methods used and results obtained in the MITC exposure studies performed after soil injection application with and without anti-drip system are described in the following.

- **Exposure of the user to methylisothiocyanate during soil decontamination with Metam-sodium on industrial potato land (Blootstelling van de toepasser aan methylisothiocyanaat tijdens de grondontsmetting met metam-natrium in het fabrieksaardappelgebied) (Mulder, A. et al 1994)**

In this study, Monam (metam sodium, 510 g/L) was applied by soil injection onto 15 different parcels as described in table B.6.15.2.2-1.

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Table B.6.15.2.2-1: Injection equipment and soil parameters for decontamination of soils with Metam sodium/MITC.

Sandy soil	Reclaimed moor soil
Injection equipment specification	
- 11 parcels decontaminated - 9 parcels treated with spade injectors: 7 with antidrip caps, 2 with blow through system (1 turned on and 1 turned off) - 2 parcels treated with cultivator injector fitted with blow through system, 1 turned off and 1 turned on - flat driven rollers - soil sealing efficiency moderate to excellent	- 4 parcels decontaminated - 3 parcels treated with spade injectors: 2 with antidrip caps, 1 with blow through system turned on - 1 parcel treated with cultivator injector fitted with antidrip caps - flat driven rollers - soil sealing efficiency moderate to good
Soil parameters	
pH: 4.4 – 5.6	pH: 4.2 – 5.8
Moisture: 13.4 – 23.7 %	Moisture: 21.5 – 32.6 %
Organic content: 4.2 – 9.1 %	Organic content: 13.3 – 28.6 %

Material and methods:

Metam-sodium (510 g/L) was applied onto 15 different parcels at 12 different field locations in the Netherlands during soil decontamination of industrial potato land in the autumn (end of September until mid of November) of 1992. Tractor-drawn equipment was used with 12 parcels treated with spade injector and 3 parcels treated with cultivator injection equipment, respectively. In order to avoid exposure during turning at the headlands all injection equipments used were fitted with an anti-drip systems, i.e. anti-drip caps or blow-through system. Irrespective of the injection equipment used all tractors were fitted with one to two flat driven rollers for sealing of the soil. Soil decontamination application was carried out by contractors taking part in the exposure measurements and all contractors were representative for the group of soil fumigants applicators as was the selected area of the parcels. Contractors wore overalls and were using gloves. During injection gloves were stored inside or outside of the tractor cabin or in the overall. Furthermore, filling times and the number of filling operations, spills during filling and number of drip during turns at the headlands were recorded.

Application rate of Monam was 300 L/ha and 11 soils were classified as sandy soils and the 4 soils were classified as reclaimed moor soils. Soil parameters such as soil temperature, pH, soil humidity and organic content were determined for each treated soil in addition to meteorological parameters such as wind speed, wind direction, air humidity and air temperature, respectively. Exposure measurements were performed on the operators, within the tractor cabins and outside of the tractor cabin near the tractor driver.

MITC concentrations were determined by using DuPont P4LC air pumps with a flow-through of 40 L/h. Pumps were connected to an SKC adsorbent tube which activated carbon as the adsorbent material. After termination of sampling adsorbent material extracted with acetone and analyzed by gas chromatography (GC).

Blank tests for determination of the MITC background concentration conducted in addition to penetration measurements for the investigation of the adsorption capacity of the adsorbent tubes used were performed for both personal and tractor cabin measurements. In addition, recovery measurements were performed to assess extraction efficiency and recovery of MITC of spiked adsorbent material. Duration of 7 h was taken into account for one representative working day.

Analytical investigations:

Recovery experiments demonstrated that average recovery of MITC in spiked acetone solutions to which the content of an SKC adsorbent tube was added amounted to 87 %.

On the basis of the analytical method used the limit of detection (LOD) was shown to be < 0.01 µg/mL in acetone as the solvent.

All blank tests performed prior to injection application of Metam-sodium on the respective fields demonstrated background values in the range of < 0.01 µg/mL to 0.08 µg/mL of acetone. There were two outliers with figures of 0.15 and 0.24 µg/mL which were due to contaminations from adjacent fields. In addition, penetration experiments did not reveal breakthrough of the adsorbent material used since no penetration of MITC to a second SKC tube could be demonstrated and MITC was efficiently adsorbed onto the activated carbon. On four parcels exposure measurements were performed in duplicate. The reproducibility of the method used was investigated in the tractor cabin air of four different parcels by performing duplicate measurements. Variations between values of duplicate measurements of the cabin air were within a range of 0.5 – 29 %.

The flow rate of the air pumps used was shown to be within 10 % of the set flow-through of 40 L/h or about 667 mL/min.

The study is accepted.

Findings:

Personal, cabin and outside air MITC exposure measurements showed that in 13 of 15 measurements exposure towards MITC was low. During soil fumigation of parcel BG2 high exposure towards MITC was found on

personal measurements while exposure figures in the cabin and outside of the tractor were comparable to all other parcels treated. The higher exposure figure was the result of filling of the tractor tanks from 200 L drums during which the operator/driver was wearing gloves and subsequent storage of the contaminated gloves in the overall pockets of the driver thereby contributing to a high exposure towards MITC during the application period.

The somewhat lower exposure figures in the cabin of the tractor were due to open cabin windows thereby lowering cabin MITC concentration by dilution with fresh air.

In parcel BG11 exceptional high MITC concentrations were found on personal and cabin measurements due to damage to one of the injector spades which were repaired on the field that was already treated with Metam-sodium. As a consequence thereof both the overall and footwear of the operator/driver became contaminated with treated soil that released MITC and which resulted in the observed high MITC exposure levels. This has been confirmed by the second period of treatment on the same parcel where comparatively low MITC concentrations were demonstrated on both personal and cabin measurements with MITC concentrations of about 200 µg/h.

Exposure figures for parcels BG12 and BG13 –which are at the same location and treated at the same date– demonstrate that although exposure figures for both personal, cabin and outside air measurements were in general low, the use of blow-through equipment reduces exposure by a factor of about 2 – 3 in the tractor cabin and by a factor of about 9 outside of the tractor which confirms the effectiveness of anti-drip equipment for exposure mitigation. In addition, it was demonstrated that there was no appreciable personal MITC exposure during loading/filling processes of the tractor tanks.

Overall it could be demonstrated that average exposure figures were comparable for personal and cabin measurements (about 145 µg MITC/h for personal measurements vs. about 141 µg/h in the cabin) while measurements outside of tractor cabin were lower (29.8 µg/h). The cause for this difference is due to higher ventilation while in the closed cabins generated MITC cannot escape which results in both higher and also comparable figures for personal and cabin exposure (table B.6.15.2.2-2).

Table B.6.15.2.2-2: Average exposure towards MITC during soil injection in µg MITC/h

Field Code	Type of injector		Area treated (ha)	Duration of measurement (min)	Inhalation exposure in µg MITC/h		
					Personal measurement	Cabin measurement	Tractor measurement
BG1	Spade inj.	Db	1.28	145	48.4	45.7	23.9
BG2	Spade inj.	Ad	3.50	250	683.1	234.1	58.0
BG3	Spade inj.	Ad	2.34	215	57.1	46.6	7.4
BG4	Cultivator inj.	Ad	5.54	245	72.4	91.6	6.4
BG5	Spade inj.	Ad	1.64	130	41.9	33.3	16.6
BG6	Spade inj.	Ad	4.84	315	107.9	104.3	21.3
BG7	Spade inj.	Ad	5.41	300	149.4	177.7	44.6
BG8	Spade inj.	Ad	5.15	310	146.4	144.4	*
BG9	Spade inj.	Db	2.70	145	121.1	122.7	68.0
BG10	Spade inj.	-db	2.44	150	130.1	128.3	61.0
BG11	Spade inj.	Ad	2.75	230	739.8	706.6	**
BG12	Cultivator inj.	Db	2.38	135	14.4	16.7	1.9
BG13	Cultivator inj.	-db	2.93	200	36.3	53.1	18.6
BG14	Spade inj.	Ad	3.93	220	146.6	122.7	*
BG15	Spade inj.	Ad	2.93	245	211.4	178.4	*
Average			3.32	216	180.4	147.1	29.8
Average (w/o BG2)			3.30	213	144.5	140.9	27.0

Ad: fitted with anti-drip caps

Db: fitted with blow-through equipment switched on

-db: fitted with blow-through equipment switched off

*: not conducted (BG8: rainy weather; BG14+15: technical difficulties in mounting the respective pump units to the tractor)

** : measurement lost due to strong vibration of the tractor during application

If the mean exposure figures obtained on a µg MITC/h basis are transformed to a daily exposure per person (working day of 7 h/d considered for exposure calculations) the average exposure for personal, cabin and tractor measurements amounted to 1.26, 1.03 and 0.21 mg MITC/person/d if all parcels were considered. This exposure is reduced to 0.69, 0.68 and 0.19 mg MITC/person/d if parcels with exceptional high exposure figures due to the presence of contaminated gloves in the tractor cabin or due to repair of the equipment are excluded from exposure measurements (table B.6.15.2.2-3).

Table B.6.15.2.2-3: Average exposure towards MITC on personal, cabin and outside air measurements during soil injection application with anti-drip equipment.

Site of measurement	MITC exposure (mg/h)	MITC exposure (mg/person/d)*	MITC exposure (mg/kg bw/d)**
Personal measurements			
all parcels	0.1804	1.26	0.018
excl. BG2	0.1445	1.01	0.014
excl. BG2 + BG11	0.0987	0.69	0.01
parcel BG2	0.6831	4.78	0.068
parcel BG11	0.7398	5.18	0.074
Cabin measurements :			
Inside :			
all parcels	0.1471	1.03	0.015
excl. BG2	0.1409	0.99	0.014
excl. BG2 + BG11	0.0973	0.68	0.001
parcel BG2	0.2341	1.64	0.023
parcel BG11	0.7066	4.95	0.071
Sum of personal + cabin measurements all parcels	0.327	2.29	0.033
Outside :			
all parcels	29.8	0.21	0.003
excl. BG2	27.0	0.19	0.0027

*: working day of 7 h/d considered for exposure calculations; **: body weight of 70 kg/person assumed.

For risk assessment purposes the measured MITC exposure levels are compared to the AOEL of MITC. For this purpose exposure of operators via the inhalation route is considered only which is justified on the mode of application and the resulting release of volatile MITC into the surrounding air only.

(calculation reviewed after Peer-Review, EFSA, 2008, see below)

Comparison of MITC exposure levels after personal and cabin measurements on a mg/kg bw/d basis with the revised AOEL of 0.004 mg/kg bw/d for MITC which was determined in the 90d dog study demonstrated that the exposure accounted for 25 % to 450 % of the AOEL when average figures or figures excluding exceptional high exposure levels were considered. In addition, storage of used gloves in the cabin or the overall pockets considerably contributes to higher personal and in part also to cabin MITC exposure levels (see parcel BG2) which can be avoided by storage of used gloves outside of the cabin during soil injection application.

If situations such as unscheduled repair of injection equipment on treated fields or storage of contaminated gloves within the tractor cabin against the label recommendations are considered exposure reaches a maximum of 1890 % of the revised AOEL. In addition, if appropriate respiratory protection such as a half-mask with combination filter A1P2 is worn during unscheduled repair procedures on the field and in the further course of the application process, this high exposure, which comprises the entire application period, is reduced to 0.074 mg/kg bw/d × 0.02 (2 % permeation through filter mask) = 0.00148 mg/kg corresponding to a maximum of about 37 % of the revised AOEL (see Table B.6.15.2.2-4)

MITC is a severe respiratory irritant and respiratory protection such as a half-mask with combination filter A1P2 should be worn during the whole process. In these conditions, operator exposure is below the AOEL (see Table B.6.15.2.2-4).

Revised calculations: already Peer-Reviewed, EFSA 2008 (see below)

Open point: 2.20 MSs to discuss the outcomes and relevance to the proposed scenarios of the 3 field studies presented

[See reporting table 2(62)].

Remarks were formulated concerning the three field studies. It may be summarised as follows:

"In the first study summarised (Mulder 1994) average exposure values are considered, excluding outliers. Due to the limited number of measurements, the approach should be discussed further."

Partly agreed with the remark, as an average on ambient air samples for 15 parcels (in duplicate on 4/15 parcels) is not particularly low for an exposure study. In 2/15 cases, high MITC levels were detected, due to contamination of the coverall or gloves with product, which was considered a realistic case. Therefore, values were also mentioned in the presence of these outliers. For these high exposures, the risk was mitigated by the wearing of a respirator with maximal protection (2% penetration), and exposure was reduced to about 37% of the proposed AOEL (adapted table from DAR: table B.6.15.2.2-4)

Adapted table B.6.15.2.2-4: MITC operator exposure estimates in open field (Mulder, 1994)

Site of measurement	MITC exposure (mg/kg bw/d)		% of AOEL	
	-RPE	+RPE	-RPE	+RPE
Personal measurements				
all parcels	0.018	0.00036	450	9
excl. BG2	0.014	0.00028	350	7
excl. BG2 + BG11	0.01	0.0002	250	5
parcel BG2	0.068	0.00136	1700	34
parcel BG11	0.074	0.00148	1850	37
Cabin measurements				
all parcels	0.015	0.0003	375	7.5
excl. BG2	0.014	0.00028	350	7.0
excl. BG2 + BG11	0.001	0.00002	25	0.5
parcel BG2	0.023	0.00046	575	11.5
parcel BG11	0.071	0.00142	1775	35.5
Outside :				
all parcels	0.003	0.00006	75	1.5
excl. BG2	0.0027	0.000054	68	1.4

- MITC levels, including worst-case results and comparison with the revised AOEL (0.004 mg/kg b.w.d) during soil injection application of Metam sodium at a rate of 3000 L/ha;
- calculations based upon working duration of 7h/d, b.w. = 70 kg; respiration rate 1.25 m³/h;
- +RPE: respirator with A1P2 filter (2% penetration)

Conclusion of the RMS:

The MITC exposure study performed after soil injection application of Metam-sodium under representative field conditions demonstrated that in some situations there was exceedance of the AOEL of MITC. Appropriate respiratory protection is recommended for the operator during the whole process.

- Exposure of the user to methylisothiocyanate during soil decontamination of bulb parcels with Metam-sodium (Blootstelling van de toepasser aan methylisothiocyanaat tijdens de grondontsmetting van bloembollenpercelen met metam-natrium) (De Rooij, M. et al, 1993)

Material and methods:

Metam-sodium (BASF monam, Aamonam, Shell-NMC and Luxan monam (Metam-sodium 510 g/L) was applied for soil decontamination purposes onto 11 representative bulb parcels during 25 July and 29 August 1992 at an application rate ranging from 420 L/ha to 700 L/ha. Four of the parcels were located in South Holland (Bloembollenstreek) and seven were located in North Holland (Noordelijk Zandgebied). The average area of the fields treated in South and North Holland was 1.1 ha and four ha, respectively, and the fields were classified as sandy soils in both areas. Soil and environmental parameters such as soil moisture content, soil and air temperature, relative humidity, wind direction and wind speed during soil decontamination of parcels in addition to general weather conditions were determined.

Eight different contractors who voluntarily took part in the exposure measurements during representative application of Metam-sodium conducted soil fumigation. Three of the eight contractors were treating two parcels each. Prior to application

of Metam-sodium the status of the contractors' personal equipment with regard to work clothes, type of footwear, gloves and facemasks was recorded. All parcels were treated using specialized equipment, i.e. cultivator injectors that inject Metam-sodium into the soil at a depth of about 18 – 20 cm. One equipment used for this purpose was fitted with a blow-through device on the injector thereby reducing the loss of Metam-sodium when turning at the headlands. Immediately after injection, one or two flat rollers one of which was a driven roller to ensure that the soil was well compressed and sealed compressed the soil. In all cases, the injection activities started on the downwind side of the parcel thereby reducing additional exposure because of emission of MITC from parcels that were already treated.

The average tank capacity of the tractors was 450 – 500 L which was filled from 3,000 L supply tanks or 200 L drums. Transfer of Metam-sodium from storage tanks or drums to the tractor tanks were accomplished by means of a positive pressure or vacuum system. Positive pressure systems are used for 3,000 L supply tanks while both systems were in place for 200 L storage containers.

The following parameters for the application of Metam-sodium on each treated parcel were recorded:

- Type and capacity of stock container
- Filling system used, injector tank capacity, number of filling procedures per parcel, filling time and total filling volume
- Injector type, working width, number of rollers, injection depth and type of caps (anti-drip caps etc.)
- Dosage applied per parcel and product used
- Working direction, number of work sessions per parcel, work duration and sealing level of soil

For the determination of exposure of MITC only the inhalation route of exposure was considered and exposure via the dermal and/or oral route of exposure was considered negligible.

Representative measurements were performed during filling to gain an impression on possible exposure during operations other than soil injection. MITC exposure measurements were performed on the operators/tractor drivers (personal measurements) and within the tractor cabin. MITC concentrations were determined by using DuPont D4LC air pumps operating at a flow-through of 40 L/h connected to an SKC adsorbent tube with activated carbon as the adsorbent material. SKC tubes were fastened to the lapel of the operators and in the tractor cabin. After termination of sampling adsorbent material extracted with acetone and analyzed by a suitable gas chromatography (GC) method with NP (nitrogen-phosphorus) detection. The robustness and stability of the method used for exposure measurements was verified by conduction of flow rate controls, blank tests for MITC background concentrations, extraction efficiency (recovery) experiments and penetration measurements to investigate the capacity of the used SKC adsorbent tubes. Within the tractor cabins MITC exposure was determined in duplicate while single determinations were carried out for personal exposure measurements. Duration of 7 h was taken into account for one representative working day.

Analytical investigations:

Recovery experiments demonstrated that average recovery of MITC in spiked acetone solutions to which the content of an SKC adsorbent tube was added amounted to 66 %.

On the basis of the analytical method used the limit of detection (LOD) was shown to be $< 0.01 \mu\text{g/mL}$ in acetone as the solvent. Blank tests performed prior to injection application of Metam-sodium on 5 representative parcels (parcels 1, 4, 8, 9 and 10) demonstrated background levels of MITC below the limit of detection ($< 0.01 \mu\text{g/mL}$). Good repeatability could be shown for the duplicate measurements within the tractor cabin since measured figures differed on average by 3.15 % only.

Penetration experiments did not reveal breakthrough of the SKC adsorbent tubes since no penetration of MITC to a second SKC tube could be demonstrated and MITC was efficiently adsorbed onto the activated carbon. MITC quantities in the first adsorbent tube were $3.2 \mu\text{g}$ and $34 \mu\text{g}$ indicating that the adsorbent capacity of one tube is high and that MITC figures obtained after work up of the tubes accurately reflect the actual airborne MITC concentrations.

The average flow rate of the air pumps used was shown to be 39.6 L/h (or 660 mL/min) which was within 1 % of the set flow-through of 40 L/h (or 667 mL/min).

The study is accepted.

Findings:

Nearly all contractors were wearing suitable and effective protective equipment, i.e. shoes or boots, overalls and gloves, during filling and soil injection of Metam-sodium. Gloves were stored outside the tractor cabin. Only one contractor working on parcel no. 2 was wearing no gloves but washing the hands immediately after filling at the company. None of the contractors used a face mask.

During the MITC measurements performed after soil injection application of Metam-sodium it could be demonstrated that in most cases obtained exposure figures for personal measurements were comparable to or slightly higher than corresponding figures for cabin measurements in the respective parcels. Only in parcels no. 5 and 6 figures for cabin measurements were higher than for personal measurements in the same parcel and it was indicated that this might have been the result of a defective injector construction. Furthermore, large differences in the measured MITC exposure levels were noted among the various parcels which might be due to a

combination of influencing factors such as wind speed and wind direction, moisture content in the soil and rate of transformation of Metam-sodium to MITC. The measurement results gave no clear indication for a connection between the level of MITC exposure and the presence of special equipment in the cabin or the type of soil sealing technique used.

In general, highest exposure figures for both personal or cabin MITC exposure were measured particularly at the end of the decontamination period when decontamination of the end lands was performed which is explainable by release of MITC from Metam-sodium that leaked on the ground of the end lands or into shallow soil when the tractor was turning.

In parcels 10 and 11 soil injection was performed by the same contractor using soil injection equipment with blow-through system. When the blow-through system was switched on considerably lower MITC exposure levels (about 6-times lower) were measured in parcel 10 as compared to parcel 11 when the blow-through equipment was not operating.

In parcel 11 leakage of Metam-sodium was reported from the injector thereby contributing to higher MITC exposure levels on personal and cabin measurements, respectively (see Table B.6.15.2.2-5).

Table B.6.15.2.2-5: Injection equipment and soil parameters for decontamination of soils with Metam-sodium/MITC. MITC exposure levels on personal and cabin measurements.

Parcel No. (Application rate L/ha)	Parameters of sandy soil	Injection equipment: cultivator injector w/o anti-drip caps	MITC concentration in air (mg/m ³)	
			Personal measurements	Tractor cabin measurements
1 (700)	moisture content: 9.4 % soil temperature (5 – 20 cm): 19-22°C Wind speed: 3-5 m/sec. air temperature (1m): 17-21°C air humidity: 81-93 %	2 flat rollers + powered rollers	0.045	0.041
2 (420)no PPE	moisture content: 12.3 % soil temperature (5 – 20 cm): 16-18°C Wind speed: 2 m/sec. air temperature (1m): 18-20°C air humidity: 51-63 %	1 flat roller + powered roller	0.006	0.006
3 (700)	moisture content: 10.3 % soil temperature (5 – 20 cm): 22-26°C wind speed: 4-5 m/sec. air temperature (1m): 25-27°C air humidity: 31-32 %	(leaks) 1 flat roller + powered roller	0.037	0.025
4 (525)	moisture content: 4.8 % soil temperature (5 – 20 cm): 17-20°C Wind speed: 3-5 m/sec. air temperature (1m): 19-22°C air humidity: 59-92 %	2 flat rollers + powered rollers	0.047	0.031
9 (560)	moisture content: 11-11.2 % soil temperature (5 – 20 cm): 17-18°C Wind speed: 4-11 m/sec. air temperature (1m): 16-19°C air humidity: not measured (defective device)	2 flat rollers + powered rollers	0.162	0.159
11 (700)	moisture content: 7.6-8.4 % soil temperature (5 – 20 cm): 17°C Wind speed: 5-6 m/sec. air temperature (1m): 19-20°C air humidity: > 90 %	2 flat rollers + powered rollers	0.068	0.066
10 (700)	moisture content: 7.6-8.4 % soil temperature (5 – 20 cm): 17°C Wind speed: 5-6 m/sec. air temperature (1m): 17-18°C air humidity: > 90 %	2 flat rollers + powered rollers	0.011	0.013

Parcel No. (Application rate L/ha)	Parameters of sandy soil	Injection equipment: cultivator injector w/o anti-drip caps	MITC concentration in air (mg/m ³)	
			Personal measurements	Tractor cabin measurements
		Injection equipment: cultivator injector + anti-drip caps		
5 (700)	moisture content: 14.5 % soil temperature (5 – 20 cm): 16-16.5°C Wind speed: 2-3 m/sec. air temperature (1m): 14-16°C air humidity: 97-98 %	1 flat roller + powered roller	0.109	0.119
6 (700)	moisture content: 12-12.5 % soil temperature (5 – 20 cm): 16.5-19°C Wind speed: 0-3 m/sec. air temperature (1m): 18-19°C air humidity: 75-82 %	1 flat roller + powered roller	0.170	0.187
7 (560)	moisture content: 10-11 % soil temperature (5 – 20 cm): 15-16°C Wind speed: 4-6 m/sec. air temperature (1m): 16°C air humidity: 74-77 %	2 flat rollers + powered roller	0.044	0.042
8 (700)	moisture content: 10.5-12 % soil temperature (5 – 20 cm): 17-22°C Wind speed: 2-4 m/sec. air temperature (1m): 16-22°C air humidity: 65-89 %	2 flat rollers + powered roller	0.105	0.070
Average parcels 1 – 11	moisture content: 9.4 % soil temperature (5 – 20 cm): 19-22°C Wind speed: 3-5 m/sec. air temperature (1m): 17-21°C air humidity: 81-93 %		0.073	0.069

*: 7 h per working day, body weight: 70 kg/person; cabin windows and doors were closed in tractors of all 11 parcels; with the exception of tractors on parcels 2 + 3, all tractors were equipped with ventilation.

Table B.6.15.2.2-6 : Results of personal and tractor cabin MITC exposure measurements after application of Metam-sodium on bulb parcels expressed as mg MITC/person/d and mg MITC/kg bw/d.

Parcel No. (Application rate L/ha)	MITC concentration in air (mg/m ³)		MITC exposure (mg/person/d)*		MITC exposure (mg/kg bw/d)*	
	Personal measurements	Tractor cabin measurements	Personal measurements	Tractor cabin measurements	Personal measurements	Tractor cabin measurements
1 (700)	0.045	0.041	0.397	0.359	0.00567	0.00513
2 (420) no PPE	0.006	0.006	0.056	0.054	0.0008	0.00077
3 (700)	0.037	0.025	0.325	0.216	0.00464	0.00309
4 (525)	0.047	0.031	0.412	0.267	0.00589	0.00381
5 (700)	0.109	0.119	0.951	1.044	0.0136	0.0149
6 (700)	0.170	0.187	1.491	1.637	0.0213	0.0234
7 (560)	0.044	0.042	0.388	0.370	0.00554	0.00529
8 (700)	0.105	0.070	0.918	0.612	0.0131	0.00874
9 (560)	0.162	0.159	1.421	1.392	0.0203	0.0199
equipment with blow-through system:						
10 (700)	0.011	0.013	0.100	0.116	0.00143	0.00166
11 (700) BT not operating	0.068	0.066	0.597	0.574	0.00853	0.00820
Average parcels 1 – 11	0.073	0.069	0.641	0.604	0.00915	0.00863

*:7 h per working day, body weight: 70 kg/person; respiration rate/person: 1.25 m³/h

Revised calculations: already Peer-Reviewed, EFSA 2008

Open point: 2.20 MSs to discuss the outcomes and relevance to the proposed scenarios of the 3 field studies presented

[See reporting table 2(62)].

“In the study by De Rooij 1993, the final conclusions are based on average exposure levels. In six out of 22 measurements, the AOEL is exceeded, whereas the exposure level is below the AOEL if the average is taken into account. Due to the limited number of measurements, the approach should be discussed further.”

Again, there were 11 treated fields, a number which is acceptable. After inspection of the high values among the operators, it would appear that exceedence (parcel 6 and 9) was about 5-5.3-fold the AOEL. Average values were up to 229% of the AOEL. As the global evaluation demonstrated the need for RPE with maximal protection, even the outliers from this study would have been exposed to MITC at levels of about 12% of the AOEL, which is acceptable

(adapted table B.6.15.2.2-7 from DAR: bold values for outliers)

Table B.6.15.2.2-7: operator exposure estimates in open field as compared to the systemic AOEL of MITC
(De Rooij, 1993)

Parcel No. (Application rate L/ha)	MITC exposure (mg/kg bw/d)		% of AOEL			
			-RPE		+RPE	
	Personal	Tractor cabin	Personal	Tractor cabin	Personal	Tractor cabin
1 (700)	0.00567	0.00513	142	128	2.8	2.6
2 (420)	0.0008	0.00077	20	19	0.4	0.4
3 (700)	0.00464	0.00309	116	77	2.3	1.5
4 (525)	0.00589	0.00381	147	95	2.9	1.9
5 (700)	0.0136	0.0149	340	373	6.8	7.5
6 (700)	0.0213	0.0234	533	585	10.7	11.7
7 (560)	0.00554	0.00529	139	132	2.8	2.6
8 (700)	0.0131	0.00874	328	219	6.6	4.4
9 (560)	0.0203	0.0199	508	498	10.2	10.0
10 (700)	0.00143	0.00166	36	42	0.7	0.8
11(700)	0.00853	0.00820	213	208	4.3	4.1
Average (1–11)	0.00915	0.00863	229	216	4.6	4.3

•MITC levels, including worst-case results and comparison with AOEL (0.004 mg/kg b.w.d) during soil injection application of Metam sodium at a rate of 420-700 L/ha;

•calculations based upon working duration of 7h/d; b.w. =70kg; respiration rate 1.25 m³/h;

•+RPE: respirator with A1P2 filter (2% penetration).

(calculation reviewed after Peer-Review, EFSA, 2008, see above)

Conclusion:

The MITC exposure studies demonstrated that after soil injection application of Metam-sodium on eleven representative fields under representative conditions the AOEL of MITC was exceeded in most of the field parcels treated when RPE was absent. Furthermore, the varying exposure levels determined after personal and tractor cabin measurements demonstrated possible variations in the degree of exposure depending on different factors such as environmental conditions and soil properties; it was demonstrated that there was no obvious dependency of MITC concentrations on Metam sodium application rates. This variation has been covered during the exposure measurements in the different parcels. It was demonstrated that in none of the operators an excess of the inhalation AOEL of MITC was observed, as long as the operator worked with a respiratory protection. Overall, it can be concluded that under these experimental conditions, operator exposure to MITC is below AOEL only with special protective equipment.

- Operator exposure to methyl isothiocyanate and methyl isocyanate, and air monitoring of MITC, MIC, CS₂ and methylamine (Links et al., 2006; TNO report)

In this study, metam sodium was applied at 300L/ha with a tractor mounted Rumpstad shear injector to approximately 5 Ha (application rate according to label 300L/ha).

During loading and application, operator exposure to MITC and MIC was measured on the inside and one on the outside of the tractor cabin. Local area monitoring for MITC, MIC and carbon disulfide and methylamine took place at different locations around the treated field, but particular downwind, during 3 days, starting at and including the period of application.

Material and methods:

The study was conducted according to protocol P6654 entitled: "operator exposure to MITC and MIC and air monitoring of MITC, MIC, carbon disulfide and methylamine following use of metam sodium of 31 October 2005. The field study was conducted between 2 and 5 November 2005.

Loading and application:

Loading operation: Metam sodium (content 42%; lot n° G5A0041796; soluble concentrate (SL)) from 210 L containers was loaded into the tank and was applied with normally used application equipment: a tractor mounted with a Rumpstad shear injector (pressure approximately 7 bars).

For each loading, 2 containers metam sodium were loaded. After loading, a small amount (less than 1L) remained in the containers. In total 8 containers of each 210 L (total amount 1680L) were loaded. The amount left in the tank of the Rumpstad after application was estimated at 180L by reading out the scale of the tank. In total approximately 1500 L of metam sodium was used for treatment of approximately 5 Ha (300L/ha).

The machine featured a working width of 3 m. Metam sodium was loaded in the tank (capacity 500 L) that was located at the front of the tractor. Via a flexible pressure tubing metam sodium was transported under pressure to the spray valves below the plough. The Rumpstad injected metam sodium in the soil at a depth of 15-20 cm, and worked the soil with a roto-tiller. After injection the soil was flattened with a roller that was part of the application equipment.

Personal exposure monitoring started just before start of loading. After applying metam sodium for approximately half an hour the operator indicated that the field was not sufficiently prepared. The application was stopped for 12 minutes to get a tractor with the appropriate equipment for preparation. This tractor preceded the tractor used for metam sodium application. The first loading started at 13H16 and application stopped at 18 h. (5H). Loading 2 containers took approximately 10 minutes each time. The operator did not take breaks.

Operator: Proper protective equipment to prevent exposure was used (i.e. neoprene gloves and a full face respirator with an appropriate cartridge ABEKP3). When seated in the tractor cabin, the operator took the protective gloves and respirator off. The field was located between Raven and Eyendorf in Germany. The size of the treated zone was 5 Ha.

The operator was 43 years and experienced in loading and application of metam sodium. He was informed in writing and by word of mouth about the objective of the study, the collection of samples and the treatment of personal data. He signed in agreement on voluntary participation prior to the experiment and performed loading and application according to his normal work practices.

Air sampling for operator exposure measurements to MITC and MIC:

During loading and application, exposure samples were taken in the breathing zone of the operator. One sample was taken in the tractor cabin, located on the left window and one sample was taken outside the tractor cabin, above the left wheel. In total 6 samples were collected, 3 for MITC and 3 for MIC.

Inhalation exposure to MITC was assessed by air sampling, using an adsorption tube consisting of a front and a back section attached to a personal air sampling pump operating at a flow rate of 250 ml/min (+/- 10%).

Inhalation exposure to MIC was assessed by air sampling, using an adsorption tube impregnated with pyridylpiperazine attached to a personal air sampling pump operating at a flow rate of 50 ml / min (+/- 10%). Flow rates were checked with calibrated rota meters.

Air sampling for environmental measurements for MITC, MIC, CS₂ and methylamine:

Measurements were performed at different locations:

Location 1 to 8 were directly bordering to the treated field.

Locations 9 to 16 were located at a distance between 100 and 225 meters from the borders of the field.

At measuring locations 1, 2, 3, 4, 6, 7, 8, 12, 13, 14 air monitoring for MITC, MIC and/or carbon disulfide and methylamine took place during 3 days, including the period of application. These 10 locations were directly bordering or downwind the treated field.

CS₂ and methylamine were measured at locations 1, 2, 3, 4. CS₂ was measured once per day. Methylamine was measured once the first day, 2 times the second day and 1 time the last day. Measurements on the first day were taken during the application period. At location 1, 2, 3, 4, CS₂, methylamine and MIC were sampled by using one air sampling pump and a triple variable flow controller.

Air monitoring for CS₂ was performed by air sampling, using an adsorption tube attached to a personal air sampling pump operating at a flow rate of 200L/min.

Environmental conditions and additional data collection:

Meteorological measurements were conducted every day a few meters north east of location 1. Every 15 minutes, temperature, relative humidity, wind speed and wind direction were measured. Wind speed and direction were taken at 1.5 m height. Cloudiness was indicated. Soil temperature was measured.

Field blank: for each substance one field blank a day was taken (n=3 per substance). During the monitoring period, the blanks were located at an uncontaminated place (approximately 1 km from the treated field). The blanks were opened but no air was sucked through the tubes.

The blanks taken in the field for CS₂, methylamine, MIC and MITC were all determined below LOD. Recoveries were 80%, 72%, and 81% for methylamine spiked with high, middle and low concentration, respectively; for CS₂ recoveries were 83%, 77% or 64% for high, middle and low concentration, respectively.

Recoveries were 76%, 78%, and 78% for MITC spiked with high, middle and low concentration, respectively; for MIC recoveries were 95%, 93% or 96% for high, middle and low concentration, respectively. All these results are considered as good recoveries.

The blanks taken in the field for CS₂, methylamine, MIC and MITC were all determined below the LOD.

Field recovery samples: a known amount of substance was added to the tubes at the laboratory prior to the field work. For each substance to be sampled, duplicate spikes per day were taken with a high, middle and low concentration (b=18/substance). The field recovery samples were located near location 1 and remained capped.

Data handling: if residue levels were less than LOQ, but above LOD, 50% of the LOQ was used in calculations and summary tables. When no detectable peak was observed in the chromatogram, a value of 100% of the LOD was used.

Inhalation exposure and environmental exposure concentration are expressed in µg/m³. The amount of substance retained in the air sampling tube is divided by the total volume of air drawn through the tube during the monitoring period.

For the calculation of the geometric mean exposure concentrations were log transformed, the average of these values was calculated and subsequently transformed back to the normal value.

Monitoring time is defined as the time between connecting the tube to the air sampling pump (start of sampling) and disconnecting the tube at the end of the monitoring period.

The methods of analysis of MITC were validated at the laboratory: a validation study was carried out to simulate trapping of MITC from air and recovery was 82% when samples were spiked with 20 ng/tube and at 90% when samples were spiked with 150 ng/tube.

For CS₂, a limited validation was carried out because the target LOQ was below the range given in the standard method.

For MIC and methylamine, standard analytical methods were used and no validation was conducted.

The study is accepted.

Findings:

The degradation products of metam sodium were determined after application in a field at different locations, from the edge of the field to a maximum distance of 225 meters.

One operator exposure sample was taken and two stationary samples (one inside and one outside the tractor) for MIC and MITC.

Recoveries were correct except for CS₂ (just below 70%) and a correction for the low recovery was not possible because all measurements were below the limit of detection.

Some air monitoring samples for MIC were taken over a longer period than the prescribed 5 hours. Because the MIC concentrations in air were low to such a degree, it did not have any influence on the calculated environmental exposure.

MIC, CS₂ and methylamine were not detected around the treated field; all values were below the limit of detection. If these degradation products were indeed formed the concentrations would at the utmost be 4 µg/m³ for MIC, 400 µg/m³ for CS₂ and 27 µg/m³ for methylamine (concentrations calculated with the LOD). It should be noted that these upper bound estimates should be considered as worst case estimates.

Air concentrations MITC ranged from the limit of detection (0.042 µg/m³) to a maximum value of 35.97 µg/m³ with a geometric mean of 0.87 µg/m³ (GM calculated with 100% LOD for concentrations <LOD, n=96). The 95th percentile was 13.22 µg/m³ and the 75th percentile 6.41 µg/m³.

No direct relation could be found between the time expired after application (with a maximum of 3 days) and the MITC air concentrations at the different locations, although the first measurements over 4 hours was at almost every location the highest. Wind direction, on the other hand, seems to be important.

During air monitoring at all three days wind direction was mainly between south and southwest. Locations 1, 2, 4 and 8, located upwind at the edge of the field, showed less environmental exposure in comparison with the downwind locations 3, 6, 7, 12, 13 and 14. Also the upwind locations 5, 9, 10, 11, 15, and 16 where limited air monitoring samples were taken, showed no detectable air concentrations. At the locations 3, 6, 12 and 13 the

MITC air concentrations increased at day 3 after a decline at day 1 and 2, which could be caused by the wind direction that turned more southwest at day 3.

At location 13, which was situated 4 meters lower than the field and at a distance of 190 meters from the field, MITC was detected all monitoring days. Soil temperature was above the lower limit (8°C) all monitoring days.

Potential operator exposure was measured during loading and application of metam sodium with a Rumpstad injector for about 5 hours. The potential exposure of the operator to MIC and the stationary measurements to MIC inside the tractor were both below the limit of detection.

Potential exposure of the operator to MITC was 76.51 µg/m³ which represents 0.00956 mg/kg bw/d. The stationary air measurement inside the tractor during these tasks was 70.33 µg/m³ which is equivalent to 0.00879 mg/kg bw/d and outside the tractor the concentration MITC were 0.107 mg/m³ representing 0.0134 mg/kg bw/d (table B.6.15.2.2-8).

(calculation revised after Peer-Review and based upon EFSA conclusions, EFSA, 2008, see below)

Table B.6.15.2.2-8: operator exposure estimates in open field (Links, 2006)

Substance	Experimental conditions	Concentration (µg/m ³)	Exposure (mg/kg bw/d)	% of AOEL
Measurements on the operator, and in- and outside tractor cabin:				
Operator personal air sample :		76.51	0.00956	239 (*4.8)
Sample taken inside tractor:		70.33	0.00879	219 (*4.4)
Sample taken outside tractor:		107.88	0.0134	335 (*6.7)
Environmental measurements:				
Location 1: 10 samples	Field boundary, upwind, measure during whole study duration	<0.042		
Location 2: 10 samples	Field boundary, upwind, measure during whole study duration	<0.042		
Location 3: 10 samples	Field boundary, downwind	4.47 – 23.71	0.000558-0.00296	14.0-74.0
Location 4: 10 samples	Field boundary, downwind, measure during whole study duration	0.07-1.82	0-0.0002275	0-5.7
Location 5	Field boundary, downwind	Pump defect, no valid measurement.	-	-
Location 6: 10 samples	Field boundary, downwind, measure during whole study duration	4.77-24.35	0.000596-0.00304	14.9-76.0
Location 7: 10 samples	Field boundary, downwind, measure during whole study duration	4.21-13.31	0.000526-0.00166	13.2-41.5
Location 8: 10 samples	Field boundary, measure during whole study duration	0.042-6.85	0-0.000856	0-21.4
Location 9: 2 samples	100 m of field, upwind	0.08	0	0
Location 10: 1 sample	100 m of field, upwind	0.09	0	0
Location 11: 1 sample	100 m of field, upwind	0.04	0	0
Location 12: 9 samples	100 m of field, downwind, measure during whole study duration	0.66-3.62	0-0.000452	0-11.3
Location 13: 10 samples	100 m of field, downwind, measure during whole study duration	2.10-13.19	0.000262-0.00164	6.6-41.0
Location 14 : 10 samples	100 m of field, downwind, measure during whole study duration	0.042-35.96	0-0.00449	0-112
Location 15: 1 sample	200 m of field, upwind	0.1	0	0
Location 16: 1 sample	200 m of field, upwind	0.09	0	0
Geometric mean		0.87	Mean: 0.00010875	2.7
Minimum – maximum:		0.042 -35.97	0-0.00449	0-112

- MITC levels, including worst-case results and comparison with revised AOEL (0.004 mg/kg b.w.d) during soil injection application of Metam sodium at a rate of 420-700 L/ha;
- calculations based upon working duration of 7h/d; b.w. =70kg; respiration rate 1.25 m³/h;
- *RPE: respirator with A1P2 filter (2% penetration).
- The measurements on locations 5, 9, 10, 11, 15, 16 were not considered in the final conclusions of the study.

Conclusion: after metam sodium application, MIC, CS₂ and methylamine were not detected around the treated field. The potential exposure of the operator to MIC and the stationary measurements to MIC inside the tractor were both below the limit of detection.

Air concentrations of MITC measured in the field ranged from the limit of detection (0.042 µg/m³) to a maximum value of 35.97 µg/m³ with a geometric mean of 0.87 µg/m³ (GM calculated with 100% LOD for concentrations <LOD, n=96). From these measurements potential operator and bystander exposure can be estimated:

Potential operator exposure measured during loading and application of metam sodium for about 7 hours was 76.51 µg/m³, which represents 0.00956 mg/kg bw/d (239% of the revised AOEL). Samples taken inside the tractor were quite similar (70.33 µg/m³ corresponding to 0.00879 mg/kg bw/d = 219% of the revised AOEL.

Bystander exposure: samples taken in the field reached a mean value of 0.87 µg/m³ corresponding to 0.000018125 mg/kg bw/d = 0.45% of the revised AOEL for a whole duration of 1 hr (the value of 1 hour for the bystander of 60 kg was agreed upon during PRAPeR meeting). As described below (B.6.15.3.1), even this maximum ambient MITC level to which a bystander would be exposed during 1 hour, would correspond to an internal dose well below the AOEL (18.73%).

These results indicated an acceptable exposure for the operator, certainly in the presence of RPE. See table B.6.15.2-8, adapted and corrected (error in average value in the original DAR also corrected).

The exposures are compared with the newly agreed AOEL for MITC (0.004 mg/kg b.w.d). Operators, wearing RPE with protection of 98% are maximally exposed to about 6.7% of the AOEL.

For information, average environmental exposures to MITC amount to about 2.7% of the AOEL for an operator exposed during 7h/d. One outlier accounted for about 112% of the AOEL. Since this value corresponded with a MITC level measured at 100m downward of the field, while other measurements downwind, even at the field boundary were lower and even <LOQ, it was considered unlikely by the expert meeting that the exposure at 0-100m of a bystander being present during 1 hour, was of meaningful concern (see also relevant calculations in B.6.15.3.1 below). Anyway, the MITC level measured in the breathing zone, inside and immediately outside the tractor is considered most relevant for the operator exposure.

B.6.15.2.3 Operator exposure towards MIC, CS₂ or methylamine after application of Metam sodium in the field (Annex IIIA 7.2.1.2)

The company addressed the possible presence of MITC, MIC, CS₂ and methylamine under field conditions at toxicologically meaningful concentrations after metam sodium applications (see study of Links, 2006).

As reported under point B.6.15.2, levels of CS₂, methylamine, and MIC at day 0-4, after application of metam sodium were <0.4 mg/m³, 0.027 mg/m³, and <0.004 mg/m³, respectively.

These results show that under realistic application conditions of metam sodium, while MIC, CS₂ and methylamine were below the limit of detection, MITC was identified at significant levels.

B.6.15.2.4 Operator exposure towards MITC after application of Metam sodium in the greenhouse (Annex IIIA 7.2.1.2)**First greenhouse trial (soil injection), submitted in the original DAR**Findings:

In the study performed by den Tonkelaar and Heidema (see point B.6.15.4.2.2 for details) it was demonstrated that MITC concentrations in the air of the greenhouse ranged from 65 mg/m³ on the day of application to 4.7 mg/m³ on day 5 after soil injection application of Metam sodium on an area of 0.123 ha at a rate of 1950 L/ha (Table B.6.15.2.4-1).

Table B.6.15.2.4-1: Course of MITC concentrations in the greenhouse during a period of 5 days after soil injection application of Metam sodium at a rate of 1950 L/ha.

Time (h)	12	24	36	40	48	54	60	72	78	84	96	108	120
MITC (mg/m ³) ¹	65	52	40	49.5	33.7	51.6	42	22.6	27.9	17.9	8.4	8.4	4.7
mg/kg bw/d	8.125			6.18			5.25						0.58
mg/kg bw /h	1.16			0.883			0.75						0.08

¹: only approximate concentrations given

For the treatment of an area of 0.123 ha in a greenhouse by soil injection application it is assumed that an operator requires about 1 – 2 h at maximum including sealing of the treated soil with a plastic foil after finishing the soil injection application.

Taking into account a concentration of 65 mg MITC/m³ in the greenhouse during application and sealing operations, a respiration rate of 1.25 m³/h, a body weight of 70 kg/person and a working time of 1 -2 h for soil injection application in the greenhouse operator exposure is calculated to be in the range of 1.16 mg/kg bw/d to 2.32 mg/kg bw/d.

This exposure exceeds the revised AOEL by a factor of about 290-580×.

If respiratory protective equipment, i.e. an A1P2 type filter mask is worn during soil injection application and soil sealing, an operator exposure of 0.023 mg/kg bw/d to 0.046 mg/kg bw/d is calculated for a working period of 1 – 2 h, corresponding with an exceedence of the AOEL with about 5.75-11.5×.

These values indicate that the use of soil injection of Metam in a greenhouse is unsafe for the operator, even when respiratory protection was present, and even for work durations as low as 2h/d, which is not covering a normal working day of 7 h/d.

Even 5 days post-application, the re-entry of a worker would be unsafe in the absence of RPE. When a mask was worn, about 40-80% of the AOEL would be consumed for a task which would last for 1-2 hours.

Table B.6.15.2.4-2: MITC operator exposure (during 1-2h) in the greenhouse at 12 – 120 h after soil injection of Metam sodium at a rate of 1,950 L/ha. and comparison with revised AOEL

Time (hr)		MITC (mg/m ³)	Exposure (mg/kg bw/1-2h)	% of AOEL
12	Without RPE	65	1.16 – 2.32	29000 - 58000
	With RPE*	65	0.023 – 0.046	575 - 1150
120	Without RPE	4.7	0.08 – 0.16	2000 - 4000
	With RPE*	4.7	0.0016 – 0.0032	40 - 80

AOEL = 0.004 mg/kg bw/d, as revised during Peer-Review; body weight: 70 kg/person

*: RPE = A1P2 filter mask (2 % penetration)

During the Peer-Review (EFSA, 2008), it was confirmed that in these agricultural conditions (soil injection of Metam in greenhouses), no safe use for glasshouse application was demonstrated (there was no information on drip irrigation available at that time).

Second greenhouse trial (drip irrigation, new study evaluated during re-submission in July 2010)

Determination of operator and worker inhalation exposure and air monitoring of methyl isothiocyanate (MITC) following drip-fumigation with Nemasol® 510 (Thouvenin, I., 2008).

Materials and Methods:

Guidelines: Protocol in compliance with following guidance documents:

- Guidance document for the conduct of studies of occupational exposure to pesticides during agricultural application (OECD EHS Publications, Series on testing and assessment n° 9, 1997).
- Guidance document on residue analytical methods, SANCO/825/00 rev. 7, 17.03.2004.

Deviations from/ remarks concerning the study protocol:

- (i) The limitation of the study resides in the fact that inhalation exposure of only one operator and one worker was monitored. However, in a soil fumigation trial, ambient air levels must also be monitored, inside the greenhouse to estimate 're-entry', and outside the greenhouse to estimate the exposure of bystander and resident. This was achieved by measuring 2 locations inside and 4 locations in the immediate neighbourhood (about 1-12m), for 4 days during and after application, and 4 locations far (about 140-220 m) from the treated greenhouse. Therefore, the study may reasonably well be considered acceptable to meet its purpose.
- (ii) The rainfall measurements of the weather station was not reliable; however, as other meteorological parameters were measured correctly (except for recording problems during 47' on d1), this has no impact on the outcome of the study.
- (iii) During travel between the field and the analytical lab, deep-freezer temperatures were out of the expected range ($-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$) occasionally. It was considered that the outcome of the study was not negatively impacted as all samples were visibly frozen at reception, and field recoveries were considered acceptable.
- (iv) Some static air samples were lost accidentally (TS-01-D1-16 found on the ground, pump off for TS-02-D1-02 (whole exposure), TS-08-D1-16 (42'), adsorbent lost for TS-02-D4-10 and TS-10-D1-18). This was not considered a major flaw as sufficient samples were left to evaluate MITC air levels.
- (v) The following remark was made by the analytical lab: "Many samples that were received on 23.05.2007 (i.e. d1-4 samples) were not thoroughly capped. In some cases the caps jumped off the tubes during sorting. It was not recorded to which samples this happened; tubes were recapped as quickly as possible". Again, field recovery measurements remained within acceptable limits, thus it may be considered that a limited amount of MITC from the tubes in the gaseous phase could have been lost, while most substance adsorbed at the charcoal was still available for extraction and subsequent measurement.

GLP status: The study is GLP.

Experimental set-up:

- Before the start of the study, the soil of the greenhouse was prepared according to GAP for planting vegetables. The soil was finely worked and three irrigation lines were installed and covered with polyethylene plastic sheet (there was no further information about the permeability of the plastic sheet for MITC).

- Metam (formulation Nemasol® SL, 510 g/L, B.n° G060701106) was applied in a greenhouse (tunnel 96×7×2.4 m) by drip irrigation. The substance was pumped through three drip irrigation lines (previously checked for water leakage) of 85 m length (255 m in total), having 3 emitting holes/m (765 drippers in total). The soil was covered by plastic sheet over a width of 0.6-0.9 m. Taking into account the estimated lateral migration of the diluted product by capillarity over a width of 0.3 m, the estimated treated area was considered 76.5 m². The recommended dose rate is 1200 L/ha, thus the calculated amount to apply was 918 L formulation. The formulation was diluted to 1% with water, and the dosing pump was calibrated to deliver this dilution (918 L of diluted product). The quantity applied was not measured but the delivered dose was calculated taking into account the pump parameters and the duration of application. The flow rate at each dripper was 1.2 L/hour, and the calculated application time was 1 hour.

- Two study subjects (operator, worker) were monitored. The ambient MITC was captured by means of sorbent tubes mounted to personal air samplers in the breathing zone during loading, monitoring during drip-irrigation (where the operator waited outside in the neighbourhood of the treated greenhouse), and working (hole-drilling).

(i) The operator (d1) was involved in the loading (opening of the drum and introduction of a sucking tube in it, in order to transfer the product through a dosing-pump), and was present during the application (outside the greenhouse). He rinsed the irrigation lines after the treatment. The total duration of exposure was 77 minutes.

(ii) The worker was involved in drilling holes manually (0.3 m from each other) in the treated soil, 18d after the start of the application. The total duration of exposure was 30 minutes.

In addition, ten static air monitoring points were chosen inside (2 locations, #1 and #2) and around (8 locations) the treated greenhouse. The static air samplers were mounted on tripods at the height of about 1.32-1.45 m. The locations of the inside and outside air samplers are depicted in the scheme on figure B.6.15.2.4-1 and are described as follows:

-Inside the greenhouse: location #1 was about 27 m from the South opening, and location #2 was at about 28 m northwards from location #1 (i.e. about one half of the width, and 1/3rd resp. 2/3rd of the length of the treated zone).

-Outside the greenhouse: #3: 1 m outside the North main opening; #4: 1.6 m on the West side of the greenhouse; #5: 5.9 m from the South opening; #6: 1.5 m on the side of the next greenhouse (about 11.5 m Eastward of the treated greenhouse); and #7-#10: four additional locations located between ca. 139 m and 219 m from the centre of the treated greenhouse.

Locations #1-#6 (in and close nearby the treated greenhouse) were monitored during 4 days after the start of treatment, while #7-10 (more remote from the greenhouse) were monitored on d1-2 only (since it was observed that already at 48h post-treatment, ambient MITC levels were <LOQ).

Figure B.6.15.2.4-1: set-up of the greenhouse trial (Thouvenin, 2008)

-Atmospheric concentrations of MITC were measured until 4 days after application, and for 24h during and after re-entry.

-Outdoor air temperature (°C) and relative air humidity (%), wind speed (m/s) and direction (°), and air pressure (mb) were recorded above soil level.

Indoor air temperature (in the greenhouse, which was the neighbour of the treated greenhouse, °C) and soil temperature (°C) at ca. 20 cm depth, were recorded. The reported data are 60' averages (5' sampling) for 4d at application, and for 24h at re-entry.

Sample collection and analysis:

-Sampling. Exposure to MITC was assessed by personal air sampling, using coconut charcoal tubes (SKC 226-16) connected to the pump by Tygon tubing. Both the personal and static air sampling pump operated at a flow rate of 250 mL/min. The adsorption tubes were replaced at about 2h- or 4h-intervals.

-Analysis. In summary, the charcoal tubes were extracted separately with CS₂ (5% v/v) in ethyl acetate. To each sample extract and each calibration solution, a known amount of internal standard (5-ethyl-N,N'-dipropyl thiocarbamate, EPTC) was added and peak ratio was used for quantification. Analysis was performed with GC-NPD (nitrogen-phosphorous detector) with two columns (one for quantification, one for confirmation of compound identity). Ten tubes, for which high amounts of MITC were measured in the front sections, were additionally analysed for MITC in the back sections, in order to examine whether substance break-through, due to inappropriately high flow rate in the tube, would have occurred.

Data analysis:

-Inhalation exposure was expressed as the time weighted average (TWA) concentration, i.e.:

$$\text{Exposure concentration MITC } (\mu\text{g}/\text{m}^3) = \frac{\text{amount in front tube } (\mu\text{g})}{[\text{average flow rate } (\text{m}^3/\text{min}) \times \text{air sampling time } (\text{min})]}$$

Quality control:

-Analysis of fortified samples sent to the field and subsequently returned with the study samples gave recoveries that confirmed the stability of MITC on the tubes during sampling, storage and shipment.

Field control tubes were spiked with either 30.4 ng (low-dose) or 2253.9 ng MITC/tube (conditions assessed: indoors, outdoor-day and outdoor-night), and an overall recovery of 78.1-93.1% (CV<20%) was obtained. It was of note that the low-level outdoors night recovery varied from 56.4-98.6% (CV=22%). This high variation was the consequence of an unexpected >LOD measurement in a control (unfortified) specimen. The recoveries were low because the residue level measured in the unfortified specimen was subtracted from the corresponding specimens at the low and high fortification level. The >LOD value of the blank remained unexplained, but after inspection of the raw data it became clear that this was a single event, considered artefactual, and without impact on the study.

Therefore, no correction was performed for the MITC results (acceptance limits: 70-110%);

-travel fortified specimens were not analysed since field recoveries were acceptable.

-Quantification and detection limits (see evaluation of validation report TNO V7111, included):

The LOQ of MITC on the charcoal sorbent tubes was 0.03 µg MITC/tube. For a sampling time of maximally 4h, this corresponds to a lowest quantifiable air level of 0.5 µg MITC/m³.

The notifier also mentions the LOD in the operator exposure study report, and defined it as the 'lowest detectable concentration in a specimen', corresponding with 11 ng/tube. In the case where the level was <LOQ or <LOD, the values were substituted by ½ LOQ or ½ LOD, respectively.

Weather conditions:

Soil temperature on d1-4 varied between approximately 20-25°C, inside air t° was 10-35°C, and outdoor air t° was 10-25°C. On d1, the weather was sunny and warm with light breeze from the North, shifting to the South in the night. The wind became moderate from the South-East on d2, the pressure felt gently and it became cloudy, and air became colder. Rain fell slowly in the morning of d2, and wind veered suddenly to the North-West remaining moderate and sweeping away the clouds as the cold front passed, on d2 at 14:00h. The pressure was at a minimum at this time and rose up rapidly afterwards. The wind was stable from the North-West on d3-4, moderate during the day and weaker at night.

The study is considered acceptable.

Findings.

-Rationale of the study design

In the present study, the inhalation exposure to MITC was monitored, during loading and application of Nemasol 510 at the intended dose rate of 1200 L a.s./ha (operator, d1) and during working (hole drilling to prepare the plant holes by a worker, d18) inside a small greenhouse (actual treated area about 76.5 m² in total). In addition, ambient air concentrations of MITC (indoors, outdoors) were measured during d1-4.

It is important to note that the application of Nemasol 510 involved drip-fumigation with a 1% dilution of the formulation. During the application both ends of the greenhouse were open to obtain a natural ventilation during the whole application phase. Covering with a plastic sheet was performed on a width of about 60-90 cm above the drip lines.

-Quality control sampling

The recovery was 78% at minimum, thus no correction for recovery was deemed necessary.

In the sampling tubes containing the highest amounts of MITC in the front section, the back sections were also analysed (n=10), and residue levels of the back section of these samples were <LOD.

In case of 'non-detects', ½ LOD was calculated as a surrogate. The fraction break-through (back section residue relative to total residue) was 0.0055-0.06%, with an average of 0.022% of total amount. Therefore, it was considered that the pump flow-rate was correct, and break-through was insignificant.

-Operator and worker inhalation exposure to MITC

In table B.6.15.2.4-3, the inhalation exposure to MITC is presented.

The TWA concentration of MITC in the breathing zone of the operator was 39.66 µg/m³ on d1, while the worker was exposed to 0.71 µg MITC/m³ on d18.

Table B.6.15.2.4-3: Inhalation exposure of the operator (d1) and the worker (d18) to MITC during and after application of Metam in the greenhouse (Thouvenin, 2008)

activity	flow rate, initial-final [average] (mL/min)	air volume (m ³)	amount (µg/ tube)	sampling duration (min)	concentration (µg/m ³)
loading	265.6 - 267.7 [266.7]	0.0205359	814.4	77	39.66
working	253.9 - 260.2 [257.0]	0.00771	<LOD (0.0055)	30	0.71

LOD= 0.011 µg/tube, LOQ=0.030 µg/tube, ½LOD or ½LOQ values were used in the calculations; Target flow rate was 250 mL/min, air volumes were calculated on actual average flow-rates.

-Air concentrations of MITC inside and outside the greenhouse

In order to make an appraisal of the ambient air concentration of MITC, a monitoring was conducted on d1-4 (6 static locations in and nearby the treated greenhouse) and on d1-2 (4 static locations far from the greenhouse).

(a) Inside the greenhouse.

The levels of MITC measured at the static sites #1 and #2 inside the greenhouse were presented as a function of the time in table B.6.15.2.4-4 and in figure B.6.15.2.4-2.

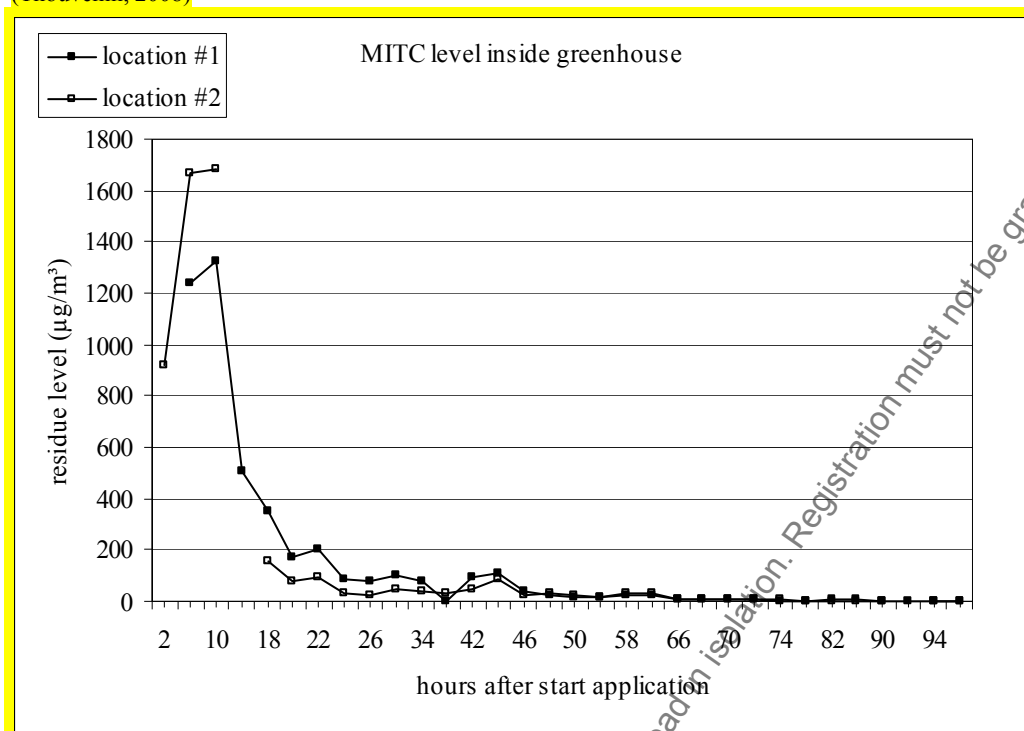
At the 2 sites, MITC was formed in considerable amounts within 2 hours from start, and a maximum peak level was already attained at 22:00h of the day of application, i.e. 10 hours from the start. The MITC concentrations amounted to about 6700 µg/m³ at that time.

The ambient MITC level dropped quickly thereafter, and small peaks were observed at resp. 30h, 44h and 58h after the start of application at both locations, where concentrations as high as 50-102 µg/m³, 86-109 µg/m³ and 23-31 µg/m³ respectively were measured.

At 66h after the beginning of the treatment, atmospheric values of MITC were <10 µg/m³.

On d4 (96h after the start of application), the MITC level inside the greenhouse was about 2-3 µg/m³.

Figure B.6.15.2.4-2 Ambient air levels of MITC inside the greenhouse, after drip-fumigation by Metam (Thouvenin, 2008)



B.6.15.2.4-4: Ambient air levels of MITC inside the greenhouse, after drip-fumigation by Metam (Thouvenin, 2008)

sample identifier	time after start of application (h)	flow rate, average (mL/min)	amount (ng/ tube)	sampling duration (min)	concentration ($\mu\text{g}/\text{m}^3$)
location #1					
TS-01-D1-16	2	252.7	66.73	106	n.a.
TS-01-D1-18	6	252.7	73108	234	1236
TS-01-D1-22	10	263.1	81297	233	1326
TS-01-D1-02	14	263.1	32464	243	507.8
TS-01-D1-06	18	257.3	20959	231	352.6
TS-01-D1-10	20	257.3	5151	115	174.1
TS-01-D1-12	22	257.3	6316	120	204.6
TS-01-D1-14	24	261.3	2790	120	88.96
TS-01-D2-16	26	261.3	2317	117	75.80
TS-01-D2-18	30	261.3	6317	237	102.0
TS-01-D2-22	34	253.0	4928	239	81.5
TS-01-D2-02	38	253.0	5.5**	238	0.091**
TS-01-D2-06	42	267.1	5932	238	93.31
TS-01-D2-10	44	267.1	3419	118	108.5
TS-01-D2-12	46	267.1	1275	119	40.12
TS-01-D2-14	48	258.9	768.6	116	25.59
TS-01-D3-16	50	258.9	491.5	116	16.36
TS-01-D3-18	54	258.9	849.0	239	13.72
TS-01-D3-22	58	249.4	1365	236	23.19
TS-01-D3-02	62	249.4	1282	242	21.23
TS-01-D3-06	66	246.7	384.0	237	6.568
TS-01-D3-10	68	246.7	158.8	119	5.410
TS-01-D3-12	70	246.7	154.8	120	5.228
TS-01-D3-14	72	258.1	141.2	118	4.638
TS-01-D4-16	74	258.1	105.3	117	3.489
TS-01-D4-18	78	258.1	171.4	239	2.779
TS-01-D4-22	82	255.4	286.8	238	4.718

sample identifier	time after start of application (h)	flow rate, average (mL/min)	amount (ng/ tube)	sampling duration (min)	concentration (µg/m³)
TS-01-D4-02	86	255.4	296.9	238	4.885
TS-01-D4-06	90	250.8	203.8	239	3.401
TS-01-D4-10	92	250.8	103.2	124	3.319
TS-01-D4-12	94	250.8	93.81	115	3.253
TS-01-D4-14	96	250.8	81.22	117	2.768
location #2					
TS-02-D1-16	2	252.7	23439	101	918.4
TS-02-D1-18	6	252.7	98811	235	1664
TS-02-D1-22	10	254.8	100638	235	1681
TS-02-D1-02	14	n.a.	n.a.	n.a.	n.a.
TS-02-D1-06	18	248.5	9201	236	156.9
TS-02-D1-10	20	248.5	2206	106	76.53
TS-02-D1-12	22	248.5	2893	120	97.02
TS-02-D1-14	24	266.1	1038	120	32.50
TS-02-D2-16	26	266.1	662.1	117	21.27
TS-02-D2-18	30	266.1	3186	239	50.10
TS-02-D2-22	34	247.2	2199	240	37.07
TS-02-D2-02	38	247.2	2007	239	33.97
TS-02-D2-06	42	253.1	2822	236	47.24
TS-02-D2-10	44	253.1	2590	119	86.00
TS-02-D2-12	46	253.1	779.6	119	25.88
TS-02-D2-14	48	246.4	890.0	116	31.14
TS-02-D3-16	50	246.4	627.8	115	22.16
TS-02-D3-18	54	246.4	912.7	239	15.43
TS-02-D3-22	58	256.7	1886	236	31.14
TS-02-D3-02	62	256.7	1759	243	28.20
TS-02-D3-06	66	249.6	394.6	236	6.699
TS-02-D3-10	68	249.6	200.9	119	6.762
TS-02-D3-12	70	249.6	214.8	120	7.173
TS-02-D3-14	72	239.4	158.5	118	5.612
TS-02-D4-16	74	239.4	112.0	117	4.000
TS-02-D4-18	78	239.4	156.1	239	2.728
TS-02-D4-22	82	252.6	214.8	238	3.572
TS-02-D4-02	86	252.6	217.5	238	3.617
TS-02-D4-06	90	258.0	155.9	238	2.538
TS-02-D4-10	92	n.a.	n.a.	n.a.	n.a.
TS-02-D4-12	94	258.0	73.03	115	2.462
TS-02-D4-14	96	258.0	65.56	117	2.172

LOD= 0.011 µg/tube, LOQ=0.030 µg/tube, **: ½LOD value was used in the calculations when <LOD;

Target flow rate was 250 mL/min; air volumes were calculated on actual average flow-rates. Gray shade: >10 µg/m³; n.a.: not available.

(b) Outside the greenhouse.

The air concentration at the proximity (1-12 m, locations #3-6, d1-4) of, and at locations more distant (140-220 m, locations #7-10, d1-4) from the treated greenhouse were monitored via static air samplers.

The air levels as a function of time are given in the tables B.6.15.2.4-5 and B.6.15.2.4-6.

The highest air concentrations were detected until 18 hours following the start of the treatment.

Peak levels of MITC were as high as about 505.8 µg/m³, in the neighbourhood of the treated greenhouse and about 7.518 mg/m³ at a larger distance. The highest MITC air concentrations were measured at location #3, which was at the North entrance of the greenhouse. Such a high value (505.8 µg/m³) could have been avoided if the tunnel would have been kept closed during fumigation.

At 96 hours post-treatment, concentration had decreased down to about 1 µg/m³ or even <LOD in the neighbourhood of the greenhouse. At greater distances, MITC levels were <LOD at all locations already 18h after the start of treatment.

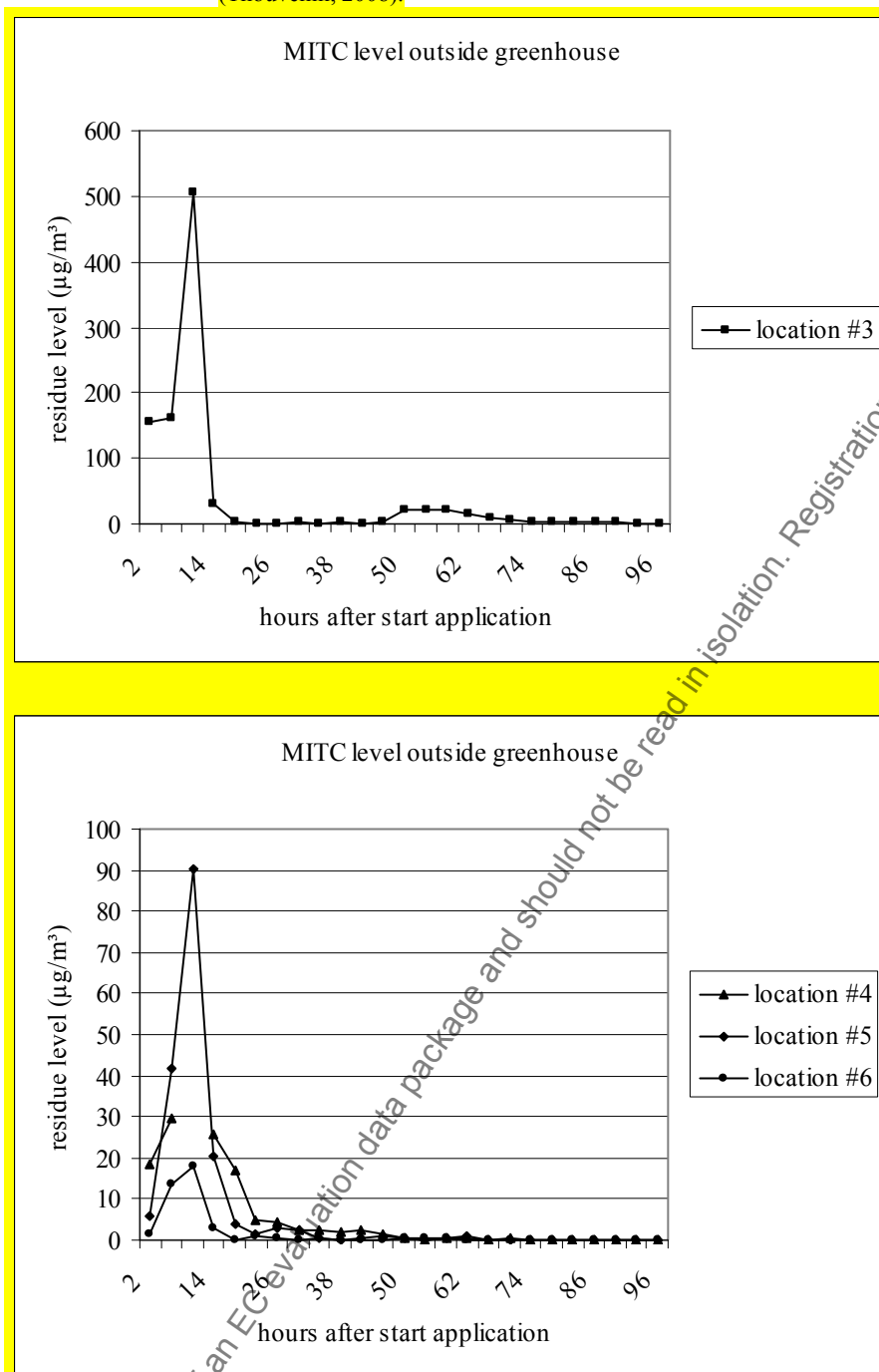
B.6.15.2.4-5: Ambient air levels of MITC (µg/m³) at the various locations outside the greenhouse (1-12 m distance), after drip-fumigation by Metam (Thouvenin, 2008)

sample identifier	time after start of application (h)	location #3	location #4	location #5	location #6
TS-0x-D1-16	2	153.9	18.52	5.701	1.289
TS-0x-D1-18	6	161.5	29.52	41.66	13.37
TS-0x-D1-22	10	505.8	(0.091**) [§]	90.26	17.80
TS-0x-D1-02	14	29.39	25.61	20.32	2.808
TS-0x-D1-06	18	2.09	17.07	3.936	0.098**
TS-0x-D1-10	22	1.116	4.913	1.226	0.9104
TS-0x-D1-14	26	0.5996	4.431	2.874	0.26*
TS-0x-D2-18	30	1.728	2.537	2.329	0.090**
TS-0x-D2-22	34	1.029	2.446	0.254*	0.243*
TS-0x-D2-02	38	2.360	1.964	0.092**	0.086**
TS-0x-D2-06	42	1.386	2.514	0.26*	0.094**
TS-0x-D2-10	46	2.652	1.514	1.039	0.236*
TS-0x-D2-14	50	21.60	0.5828	0.26*	0.26*
TS-0x-D3-18	54	22.50	0.086**	0.092**	0.25*
TS-0x-D3-22	58	20.20	0.5818	0.25*	0.5989
TS-0x-D3-02	62	14.53	0.6488	0.8013	0.23*
TS-0x-D3-06	66	8.427	0.095**	0.093**	0.098**
TS-0x-D3-10	70	6.263	0.25*	0.088**	0.092**
TS-0x-D3-14	74	3.850	0.091**	0.094**	0.10**
TS-0x-D4-18	78	3.007	0.089**	0.091**	0.10**
TS-0x-D4-22	82	2.691	0.089**	0.24*	0.094**
TS-0x-D4-02	86	2.982	0.24*	0.088**	0.093**
TS-0x-D4-06	90	1.605	0.092**	0.098**	0.090**
TS-0x-D4-10	94	1.039	0.092**	0.098**	0.090**
TS-0x-D4-14	96	1.045	0.19**	0.20*	0.18**

LOD= 0.011 µg/tube, LOQ=0.030 µg/tube, **: ½ LOD or *: ½ LOQ values were used in the calculations when <LOD or <LOQ, respectively;

[§]: as the level was unexpectedly low, when compared to that of the previous and following 4h-sampling (see also figure B.6.15.2.4-1), RMS questioned the validity of this measurement, and preferred to disregard this value in the calculation of the mean.

Figure B.6.15.2.4-2 Ambient air levels of MITC outside the greenhouse (1-12m), after drip-fumigation by Metam (Thouvenin, 2008).



In order to estimate the exposure for the bystander, the average MITC level during a certain sampling period, could be taken into account. A multi-directional average was calculated by the notifier for each of the sampling times 2h, 6h, 10h and 14h after the start of treatment. A bystander could be present during 60' at any time during the application and up to 14h after the start of the treatment, in a reasonable worst-case scenario. However, as the set-up of the trial was relatively limited (only one operator and one worker, and 8 outside static samplers monitored), it was proposed by RMS also to consider the maximal measurements in the risk assessment.

For the outdoor monitoring in the vicinity of the treated greenhouse, the average value of 204.6 µg/m³, and the maximal value was 505.8 µg/m³ was considered (table B.6.15.2.4-6). At location #4, the sample obtained at 10h after the start of treatment was unexpectedly low. A value <LOD for the whole sampling time (4h) was not

very plausible, as the previous and following sample indicated a MITC level of respectively about 30 µg/m³ and 26 µg/m³. Moreover, as MITC air concentrations were at their highest at the other locations during this sampling period, the low value remained unexplained, and RMS questioned the validity of this measurement. Therefore, it was proposed not to include this value in the calculation of the mean.

Table B.6.15.2.4-6: Ambient air levels of MITC (µg/m³) outside the greenhouse (1-12 m distance), after drip-fumigation by Metam, including average values per sampling period 2-14 h (Thouvenin, 2008)

time after start of application (h)	location #3	location #4	location #5	location #6	average	± s.d.
2	153.9	18.52	5.701	1.289	44.85	73.06
6	161.5	29.52	41.66	13.37	61.51	67.66
10	505.8	(0.091) [§]	90.26	17.8	204.62	263.33
14	29.39	25.61	20.32	2.808	19.53	11.75

§: as the level was unexpectedly low, when compared to that of the previous and following 4h-sampling, RMS questioned the validity of this measurement, and preferred to disregard this value in the calculation of the mean.

(ii) At the more remote locations (relevant for the resident exposure), an average value of 3.595 µg/m³ MITC was calculated, while the maximal value was 7.518 µg/m³.

Table B.6.15.2.4-7: Ambient air levels of MITC (µg/m³) at the various locations outside the greenhouse (140-220 m distance), after drip-fumigation by Metam (Thouvenin, 2008)

sample identifier	time after start of application (h)	location #7	location #8	location #9	location #10
TS-0x-D1-16	2	0.17**	0.21**	0.14**	0.14**
TS-0x-D1-18	6	1.246	1.633	0.26*	n.a.
TS-0x-D1-22	10	7.518	0.26*	2.080	4.523
TS-0x-D1-02	14	0.084**	0.10**	0.089**	0.5415
TS-0x-D1-06	18	0.099**	0.096**	0.10**	0.26*
TS-0x-D1-10	22	0.094**	0.090**	0.095**	0.090**
TS-0x-D1-14	26	0.092**	0.089**	0.091**	0.093**
TS-0x-D2-18	30	0.088**	0.085**	0.087**	0.089**
TS-0x-D2-22	34	0.091**	0.095**	0.088**	0.090**
TS-0x-D2-02	38	0.087**	0.090**	0.085**	0.086**
TS-0x-D2-06	42	0.098**	0.097**	0.098**	0.11**
TS-0x-D2-10	46	0.089**	0.082**	0.085**	0.094**
TS-0x-D2-14	50	0.097**	0.097**	0.096**	0.094**

LOD= 0.011 µg/tube, LOQ=0.030 µg/tube, **: ½ LOD or *: ½ LOQ values were used in the calculations when <LOD or <LOQ, respectively;

Table B.6.15.2.4-8: Ambient air levels of MITC (µg/m³) outside the greenhouse (140-220 m distance), after drip-fumigation by Metam, including average values per sampling period 2-14 h (Thouvenin, 2008).

time after start of application (h)	location #7	location #8	location #9	location #10	average	± s.d.
2	0.17	0.21	0.14	0.14	0.165	0.033
6	1.246	1.633	0.26	n.a.	1.046	0.708
10	7.518	0.26	2.08	4.523	3.595	3.145
14	0.084	0.1	0.089	0.5415	0.204	0.225

-Exposure data in relation with the proposed AOEL

Taking into account the proposed AOEL value of MITC (0.004 mg/kg b.w./d), the inhalatory exposure of the operator, the worker, bystander and resident was evaluated.

A default ventilation rate of an adult of 0.021 m³/minute was used (1.25 m³/hour, assuming moderate duty tasks). A default body weight of 70 kg was assumed for the operator and 60 kg for the other (as agreed on in the former PRAPeR meeting).

The total exposure in gramme compound was obtained by multiplying the air concentration (in g/m³) with the inhaled volume (in m³).

The inhalation exposures were calculated without and with RPE for operator and worker, and without RPE for bystanders and residents. The RPE consisted of respirators with organic vapour filter, conferring a 98%

protection (respirator with A1P2-filter). For bystander and resident, average as well as maximal MITC levels were considered in the calculation.

(i) Operator

The inhalatory exposure of the *operator* in the absence of a 98% RPE represented 26.5% of the AOEL for the operator. In the presence of RPE, about 0.53% of the AOEL would be used up. Extrapolated for a whole working day (7h), the exposure would account for about 145% of the AOEL in the absence, and 2.9% of the AOEL in the presence of RPE. Under the agricultural practice of the current trial, the exposure would thus only be safe for the operator in the presence of RPE.

(ii) Worker

The inhalatory exposure of the *worker* during hole drilling of the treated soil 18d later represented 0.18% of the AOEL, in the absence of RPE. If the cultivation would be performed with a respirator, then about 0.0037% of the AOEL of MITC would be attained.

The extrapolated value for a whole working day is also well below the AOEL, both in the absence (2.96%) and in the presence (0.06%) of RPE.

Under GAP, no re-entry of the operator is expected before day 18 after the start of treatment. Obviously, MITC level in the greenhouse remained quite high up to 66h after the start of the drip-irrigation. The highest MITC concentration was observed 10h after the start, when 1.681 mg MITC/m³ was measured.

In the event of an occasional re-entry shortly after treatment for 15', taking into account a respiration rate of 0.021 m³/min, the amount of breathed air would be 0.315 m³. With a maximal ambient MITC level of 1.681 mg/m³ (see above table B.15.2.4-3), the absorbed dose would amount to 0.52952 mg (1.681 mg/m³ × 0.315 m³). With a standard b.w. of 60 kg, the inhaled dose would be 0.00883 mg/kg b.w./d, and corresponds with about 2.2× the AOEL of MITC, without respiratory protection.

In the presence of RPE with 98% protection, a safe re-entry is possible, with an estimated exposure of 0.00018 mg/kg b.w./d, corresponding with 4.4% of the AOEL. An occasional re-entry in the greenhouse within the first 24h after the treatment is considered unsafe in the absence of suitable respiratory protection.

(iii) Bystander

The *bystander*, present during 60' in the vicinity of the greenhouse (1-12m) at a moment when the average outdoor concentration of MITC was at its highest (204.62 µg/m³), would be exposed to a MITC level corresponding to about 107% of the AOEL. Another approach could be to rely on the average value of ambient MITC levels from 2h-14h post-treatment at the critical location (at the level of the open entrance of the tunnel). With a mean level over this time-lapse of 213 µg/m³, the intake for a bystander during one hour would be 0.0044 mg/kg b.w./d, corresponding with about 111% of the AOEL, which is comparable with the multi-directional average.

However, it could be remarked that the exposure should take into account the maximal MITC level, which was measured at location #3, at 10h after the start of the treatment. With a maximal ambient MITC level of 0.5058 mg/m³, and considering a breathed volume of 1.25 m³/h, the absorbed dose would amount to 0.63225 mg ÷ 60 kg = 0.01054 mg/kg b.w./d, and would correspond with about 2.6× the AOEL of MITC. If this extreme worst-case approach would be followed, then no safe use would be anticipated for the bystander.

(iv) Resident

A *resident*, could be present at a location more distant from the greenhouse (>120 m). When the average outdoor concentration of MITC was at its highest (3.595 µg/m³) during 24h, a resident would then be exposed to a MITC level corresponding with about 45% of the AOEL. The exposure could thus be considered safe for the resident.

It could be remarked that the exposure should take into account the maximal MITC level, which was measured at location #3 at 10h after the start of treatment. With a maximal ambient MITC level of 0.00752 mg, the absorbed dose would amount to 0.2256 mg ÷ 60 kg = 0.00376 mg/kg b.w./d, and would correspond with about 94% the AOEL of MITC, which is still acceptable.

The calculation of the risk assessment is shown in detail in table B.6.15.2.4-9

Table B.6.15.2.4-9: Total exposure values of MITC during and after application of Metam in greenhouse, in relation to the proposed AOEL (Thouvenin, 2008).

	operator (d1)		worker (d18)		bystander (d1)		resident	
	study	7h-day	study	8h-day	1h	1h	24h-day	24h-day
					mean	max.	mean	max.
concentration (mg/m ³)	0.03966		0.00071		0.20462	0.5058	0.003595	0.00752
sampling time (min)	77	420	30	480	60	60	1440	1440
inhaled volume (m ³)	1.604	8.75	0.625	10.00	1.25	1.25	30	30
exposure (mg)	0.06362	0.34703	0.00044	0.0071	0.255775	0.63225	0.10785	0.22554
exposure (mg/kg b.w.)	0.001060	0.005784	0.0000074	0.000118	0.0043	0.01054	0.00180	0.00376
% AOEL, no RPE	26.5	145	0.18	2.96	107	263	45	94
% AOEL, RPE (98%)*	0.53	2.9	0.0037	0.06	-	-	-	-

Values for the conditions of the study and for a full working day (8h exposure of operator or worker). For the bystander, presence during 60' in the vicinity of the greenhouse is expected; a resident, living at a distance >120m from the greenhouse may be exposed for 24h.

Parameters used for calculations: respiration rate of 1.25 m³/h and a default body weight of 70 kg (operator) or 60 kg (other);

*: Respiratory protective equipment: mask with organic vapour filter (2% penetration); -: RPE not applicable;

AOEL (MITC)= 0.004 mg/kg b.w./d

Summary and conclusions:

In the small-scale greenhouse study, a 1% dilution of Nemasol 510 g/L was applied on a surface of 76.5 m² (the actually treated surface in a small greenhouse of approximately 6-7 are) at the dose of 1200 L/ha by means of drip-irrigation.

The exposure to the main degradation product MITC was investigated. This included the risk assessment of the operator during loading and application on d1, of the worker on d18, of a bystander present in the vicinity of the greenhouse during 60', and of a resident, present during 24h at remote distances from the treated greenhouse.

The inhalation exposure of the operator and the worker, was assessed by measuring MITC levels in the breathing zone, and the value was extrapolated for a full 7h working day.

Bystander and resident exposure was assessed, taking into account the average MITC level measured on static air samplers in a period where outdoor peak-levels were detected (2-14h), in a worst-case scenario.

-The exposure of the **operator** was only acceptable in the presence of RPE, conferring a 98% protection. In the latter case, inhalatory exposure accounted for about 2.9% of the AOEL. The exposure was not safe in the absence of RPE. An occasional re-entry before d18 also requires the wearing of RPE.

-The exposure of the **worker**, re-entering the greenhouse after 18 days, in order to drill plant holes in the treated bands, was acceptable, even in the absence of RPE (exposure accounting for 2.96% of the AOEL in the absence of RPE, and 0.06% in the presence of RPE).

-The exposure of a **bystander**, present in the vicinity (1-12 m) of a treated greenhouse during 60' in a period spanning 2-12h post-treatment, was at the level of the AOEL, with an exceedence by only 107% if average MITC levels were considered over the different locations.

If the maximal value was considered (at one location at 10h post-treatment), then the bystander exposure was unsafe, as the bystander exposure was as high as 252% of the AOEL.

-The exposure of a **resident**, being present at locations more distant (140-220 m) from the treated greenhouse during 24h, represented 45% of the AOEL and was considered acceptable. If the maximal value was considered (at one location at 10h post-treatment), then the bystander exposure corresponded with 94% of the AOEL.

It was of note that not all risk-mitigating measures were undertaken during this trial. For example, the entrances of the greenhouse/tunnel were not closed during and after the treatment. Therefore, it is quite probable that the exposure of the bystander in the neighbourhood of the treated greenhouse at 2-14h after the start of treatment could be reduced, if MITC would be contained into the greenhouse during that lapse of time.

Validation of MITC analysis in air:

Reference: Validation of an analytical method for the determination of MITC of coconut charcoal air sampling tubes (Schouten, 2007 – TNO Report No. V7111).

GLP: GLP-compliance stated

Principle of the method:

After suction of air under prescribed conditions, coconut charcoal tubes are extracted with ethyl acetate with 5% carbon disulfide. The extracts are analysed by GC-NPD on two columns: one column (A) used for quantification (RTX-200, 30mx0.25mm, 0.25µm film) and the other (B) for confirmation of compound identity (CP Sil 5CB, 25mx0.25mm, 0.4µm film). An internal standard EPTC (5-ethyl-N,N-dipropyl thiocarbamate) was added and peak ratio was used for quantification.

Findings :

Specificity – interferences :

No chromatographic interferences; Blank values <30% of LOQ (demonstrated by representative chromatograms); Confirmation of identity by using second GC column of different polarity (CP Sil 5CB)

Linearity :

Linear range 1.7 – 163 µg/L (corresponding to approximately 10 – 978 ng/tube); $r^2 > 0.999$ ($n \geq 7$); Calibration graphs provided.

Recovery – precision :

-Desorption efficiency: average 86.5% (RSD=3-7%);
-Storage stability: fortified gas adsorption tubes stored at <-18°C are stable for at least 2 months. Acceptable recovery was demonstrated: after 14 days storage mean recoveries: 80-104%; after 62 days storage mean recoveries: 91-102%.
-Back section of the fortified tubes was analysed after air passage. No significant breakthrough was observed.
-Validation recovery data for MITC (corrected for desorption efficiency and small blank values): see Table B.5.3.3-1

Limit of quantification (LOQ) :

MITC: 0.5 µg/m³ air (4 h worst case sampling); 0.25 µg/m³ air (8 h ambient sampling)

Table Table B.6.15.2.4-9: Validation data for GC-NPD method for the determination of MITC in air (Schouten, 2007 – Report No. V7111)

Matrix / sampling conditions	Analyte	Method	Fortification level [ng/charcoal tube]	GC-column ^a	Recovery range [%]		RSD	
					mean	range	[%]	n
Air (worst case conditions: 35°C, 80% RH, 4 hrs at 250 mL/min)	MITC	GC-NPD	30*	A	60	55-67	8	5
				B	68	64-72	5	5
			225	A	65	63-68	3	5
				B	73	72-76	2	5
			2254	A	79	76-83	3	5
				B	81	80-82	1	5
Air (ambient conditions: 19°C, 44% RH, 8 hrs at 250 mL/min)	MITC	GC-NPD	30*	A	89	82-94	5	5
				B	89	83-96	6	5
			225	A	97	89-104	7	5
				B	95	93-97	2	5
			2254	A	97	91-105	6	5
				B	99	97-101	2	5

*Limit of quantification: 30 ng/tube (= 0.5 µg/m³ for 4h (worst case) sampling ; = 0.25 µg/m³ for 8h (ambient) sampling);

^a Column A: RTX-200; Column B: CP Sil 5 CB

B.6.15.2.5: Summary of operator exposure measurements and estimation of exposure to MITC (Annex IIIA7.2.1.2.)**(i) FIELD studies**

The results reported in the table B.6.15.2.2 show the variations in the degree of exposure depending probably of environmental conditions and soil properties; it was demonstrated that there was no obvious dependency of MITC concentrations on metam sodium application rates.

The results demonstrate that exposure to MITC during application of metam sodium under field conditions could be sometimes higher than the AOEL when no respiratory protections are worn. Therefore, exposure is always lower than the AOEL when respiratory protections are worn as recommended by the label.

Revised summary: already Peer-Reviewed, EFSA 2008

(calculation revised after Peer-Review and based upon EFSA conclusions, EFSA, 2008, see below)

Table B.6.15.2.5-1:

Summary of operator exposure to MITC using the experimental results reported in the different FIELD studies.

Exposure to MITC	Operator exposure				Experimental conditions
	mg/kg bw		% AOEL		
	-RPE	+RPE	-RPE	+RPE	
Mulder et al., 1994					Metam sodium, 510 g/L, 15 parcels, soil decontamination, tractor drawn equipment, spade injector, rollers for sealing soil. Contractors wore overalls+ gloves; 300L/ha
Personal measures	0.018	0.00036	450	9.0	
Cabin measures	0.015	0.0003	375	7.5	
Outside air	0.003	0.00006	75	1.5	
De Rooij et al., 1993					Metam sodium, 510 g/L, soil decontamination, 11 parcels(1.1-4 Ha), 8 contractors; 420-700L/ha; Contractors were wearing shoes or boots, overall and gloves
Personal measures	0.00915	0.00018	229	4.6	
Cabin measures	0.00863	0.00017	216	4.3	
Outside air	-	-	-	-	
Links et al., 2006					Metam sodium, 1 field of 5 Ha; 1500L metam sodium applied; 1 contractor, Rumpstad shear injector; no specific protections
Personal measures	0.00956	0.00019	239	4.8	
Cabin measures	0.00879	0.00018	220	4.4	
Outside air	0.0134	0.00027	335	6.7	

• MITC levels, including worst-case results and comparison with revised AOEL (=0.004 mg/kg b.w./d) during soil injection application of Metam sodium

• calculations based upon working duration of 7h/d; b.w. =70kg; respiration rate 1.25 m³/h;

• +RPE: respirator with A1P2 filter (2% penetration).

(ii) GREENHOUSE studies

- In the first greenhouse study (soil injection), air concentration just after application of metam sodium reaches 65 mg/m³ making operator exposure much higher than the revised AOEL (up to about 580× higher). In these conditions, wearing of RPE reduced the exposure but not sufficiently to reduce exposure below the AOEL (up to 11.5× AOEL). In conclusion, no safe use for glasshouse application was demonstrated (there was no information on drip irrigation available at that time).

- In the second greenhouse study (drip irrigation), the exposure of the operator was acceptable in the presence of RPE, conferring a 98% protection. In the latter case, inhalatory exposure accounted for about 2.9% of the AOEL. The exposure was not safe in the absence of RPE (145% of the AOEL reached). An occasional re-entry before d18 also requires the wearing of RPE.

In conclusion, the use of Metam applied by drip-irrigation in greenhouses was considered safe for the operator, if RPE was worn.

B.6.15.3 Estimation of bystander exposure (Annex IIIA 7.2.2)**B.6.15.3.1. Estimation of bystander exposure towards MITC after field application of Metam sodium (Annex IIIA 7.2.2)**

Due to the intended application of the soil fumigant Metam-sodium by means of soil injection or drip irrigation and due to the low vapor pressure of the active substance inhalation and dermal exposure of bystanders towards the active substance Metam-sodium is not expected.

Inhalation exposure of bystanders might occur for MITC, the volatile degradation product of Metam-sodium. MITC concentrations in air can be measured directly after the application of Metam-sodium, but no high MITC concentration in air is expected following the application of Metam-sodium due to the intended application by soil injection including soil compression or drip irrigation under a plastic film, which represents coverage comparable to a soil layer or soil seal.

- Operator exposure to methyl isothiocyanate and methyl isocyanate, and air monitoring of MITC, MIC, CS₂ and methylamine (Links et al., 2006; TNO report)

Part of the results from the studies performed for operator exposure assessment (a part of the table B.6.15.2-8 is reported here under table B.6.15.3.1-1), can be used for bystander exposure:

In the study of Links et al (2006), MITC was measured in air at a distance of 0 to 200 m of the field during a period of 0-4 days after application. In this study, a worst-case situation is represented by 36 µg/m³ MITC, giving an exposure of 6.4.10⁻⁴ mg/kg bw/hour. The corresponding exposure contributes to 16% of the AOEL agreed during PeerReview.

Revised table: already Peer-Reviewed, EFSA 2008

(calculation revised after Peer-Review and based upon EFSA conclusions, EFSA, 2008, see below)

Table B.6.15.3.1-1: Air concentrations of MITC at operator level during application of Metam

Substance	Experimental conditions	Concentration in µg/m ³	Exposure (mg/kg bw/1 h)	% of AOEL agreed during the Peer-review
Environmental measurement:				
Location 9: 2 samples	100 m of field, upwind	0.08	0	0
Location 10: 1 sample	100 m of field, upwind	0.09	0	0
Location 11: 1 sample	100 m of field, upwind	0.04	0	0
Location 12: 9 samples	100 m of field, downwind, measure during whole study duration	0.66-3.62	0 – 7.5×10 ⁻⁵	0 – 1.89
Location 13: 10 samples	100 m of field, downwind, measure during whole study duration	2.10-13.19	4.38×10 ⁻⁵ – 2.75×10 ⁻⁴	1.1 – 6.87
Location 14: 10 samples	100 m of field, downwind, measure during whole study duration	0.042-35.96	0 – 7.5×10 ⁻⁴	0 – 18.73
Location 15: 1 sample	200 m of field, upwind	0.1	0	0
Location 16: 1 sample	200 m of field, upwind	0.09	0	0

Personal measurements, stationary measurements in and outside tractor. Mean soil temperature: 8.8-12.1°C and air temperature: 7.9-18.9°C. Exposure duration of 60 minutes assumed; respiration rate 1.25 m³/h; bodyweight: 60 kg/person

From the open literature:

- Methyl isothiocyanate volatilization from fields treated with metam sodium (Saeed et al, 2000)

Material and methods:

Field conditions and fumigant application:

This study was conducted in four fallow fields intended to be grown with potatoes. Methyl isothiocyanate air concentrations were measured above these fields following application of metam-sodium (345 g a.i./kg) either through injection using a ground rig (fields 1 and 2) or by chemigation using the center-pivot irrigation systems (fields 3 and 4). Both methods are used commercially in Wisconsin for the application of the fumigant and are claimed to be equally effective. The study fields were 2±5 miles apart and surrounded by similar fields. The soil of the study fields was Plainfield loamy sand.

None of the adjacent fields was fumigated during the time of the studies, thus minimizing background MITC air concentrations.

Solutions of metam-sodium (345g a.i./kg) were injected in the soil at a rate of 480litre/ha (166 kg/ ha) in fields one and two using a ground applicator with knives or shanks spaced 15cm apart. The chemical was mixed with an equal volume of water in the tank before injection. The knives were pulled through the soil at a depth of 25cm and the fumigant was released from ports behind each shank at depths of 5, 15 and 25cm. A soil compactor was connected to each rig to compact the soil over the furrow made by the injection shanks.

In fields three and four the fumigant solution at a rate of 480 liter/ha was mixed with 169m³/ha of irrigation water through a metering pump that was connected to the center-pivot irrigation system. Chemigation in both fields was performed during the night hours and completed in the early morning hours of the sampling day.

Method of estimation:

Immediately following fumigant application to each of the four fields, equipment for measuring MITC air concentration, temperature and wind speed was installed and measurements were started. Methyl isothiocyanate concentration in the air above treated fields was determined by drawing air into double-trap scrubbers placed on masts. The scrubbers were attached to the mast at 3 heights, 10, 100 and 200cm above the soil surface.

In each of the four fields, three masts were used. Air was drawn from the atmosphere directly into the traps at a rate of 1 liter/min. Flow meters equipped with needle valves (model Q603, Matheson Tri Gas, Parsippany, New Jersey, USA) were installed in the collection lines near the traps. The trap consisted of two Teflon-lined rubber-stopped 4-oz glass jars containing ethyl acetate (40ml). A high-volume air-sampling pump modified with a nine-inlet manifold was connected to the sampling jars via Teflon and Tygon tubing. In all the fields, most of the measurements were carried out during the daylight hours with a few night readings.

Old traps were replaced by fresh ones at the end of each sampling period. Collected MITC samples were stored in dry ice, shipped to the laboratory, and stored at 15°C until analyzed. Hourly wind speed averages were recorded at the three heights at which MITC air concentrations were measured. Hourly air and soil temperatures were also recorded. A 21X data logger (Campbell Scientific, Inc, Logan, Utah, USA) was used with thermocouples and cup anemometers to make the meteorological measurements.

Method of detection:

A gas chromatographic method reported earlier was modified and used to measure MITC concentrations in the trapped volatiles. A Varian 3700 gas chromatograph (GC) equipped with an electron-capture detector was used. The column was a 1.8m×6mm ID glass column packed with 4% SE-30/ 6% OV-210. Nitrogen. Hourly averages of wind speed at 10, 100 and 200cm heights during three days following fumigation. (b) Hourly averages of air and soil temperatures at 10cm depth during three days following fumigation. Nitrogen (99.999% pure) was used as carrier gas at 30ml/ min. The temperatures used were 90°C for the column, 260°C for the injector and 275°C for the detector. Two micro liters of MITC sample in ethyl acetate from scrubbers were directly injected into the GC. After every two-sample run, an appropriate standard was injected and the concentration of each sample was obtained from calibration curves of integrated peak area versus concentration. Detection efficiency was determined for the GC method and data were used to correct MITC air concentrations.

Estimation of collection efficiency:

Calibration trials were carried out in the laboratory to estimate MITC collection efficiencies of the scrubbers. Three scrubbers fitted with four traps of ethyl acetate each were connected to a Mason jar containing MITC-treated soil. Each Mason jar was fitted with a one-way valve to allow airflow to the jar without MITC loss. The MITC application rate to the soil in these jars, the airflow rate, and the volume of solvent in the traps were similar to those used in the field experiments. The average percentages of MITC collected in the first, second, third and fourth traps were 68, 18.9, 11.0 and 1.6, respectively. The collection efficiency was calculated as the percentage of MITC collected in the first and second traps relative to the total amount collected in all four traps. The mean collection efficiency was 87 (±3) percentages. MITC volatilization data from all four fields were corrected for collection efficiency accordingly.

The sensitivity and precision of MITC analysis were measured using spiked MITC samples of known concentration ($0.1 \pm 60 \text{ mg/ml}$). Three spiked samples were used for each concentration level. Calibration curves were generated from average responses in peak area from each concentration versus the amount of chemical spiked.

Findings:

The focus of this study was to estimate the loss of MITC by volatilization when metam-sodium was applied through either chemigation or injection to arable fields. Methyl isothiocyanate is the principal product of the transformation and constitutes about 90% of the total amount of metam-sodium applied to soils. This study assessed the potential inhalation exposure to MITC during the re-entry period.

MITC air concentrations collected at three heights above the treated fields showed distinct concentration gradients, with the highest concentrations closest to the soil surface and the lowest at the 200cm level. Above all four fields, average MITC air concentrations were higher during the first 8h following application of metam-sodium regardless of the application method. MITC air concentrations decreased with time thereafter at variable rates above the different fields.

On most sampling occasions, higher MITC emissions were observed in chemigated than in injected fields. The highest MITC concentrations were 11.2 and $7.4 \mu\text{g/m}^3$ recorded 10cm above the ground 6-8h following application and the lowest concentrations were 0.7 and $0.2 \mu\text{g/m}^3$ observed at 200cm 30 and 35h after application above chemigated and fumigated fields, respectively. One nighttime measurement was taken in each of fields 1 through 3 while three nighttime measurements were taken in field 4. MITC air concentrations observed during these periods were not significantly lower than the average of those measured during the daytime hours in three out of the four fields (Table B.6.15.3.1-2).

Table B.6.15.3.1-2: MITC air concentration measured at 10, 100 and 200 cm above the 4 treated fields.

Field/time after application (h)	MITC air concentration ($\mu\text{g/m}^3$)		
	200 cm	100 cm	10cm
Injected field 1: 166 kg/ha (480L)			
5.3	1.1	1.2	1.3
11.4	1.3	1.4	1.6
29.4	0.9	1.0	1.1
78	0.7	0.8	0.9
Mean:	1.0	1.1	1.22
Injected field 2: 166 kg/ha (480L)			
4	0.4	0.9	7.0
6	0.9	1.3	7.4
11	0.4	0.5	5.9
27.5	0.3	1.0	1.0
29.5	0.5	1.6	1.6
34.5	0.2	0.4	0.4
Mean:	0.45	0.95	3.88
Chemigated field 3: 166 kg/ha (480L) diluted in 169 m^3 water/ha			
3	1.6	2.0	3.5
6	0.9	1.3	2.8
8.4	2.0	3.0	11.2
10	1.2	1.3	4.0
14	0.7	0.8	1.7
Mean:	1.28	1.7	4.64
Chemigated field 4: 166 kg/ha (480L) diluted in 169 m^3 water/ha			
6.3	4.6	5.9	7.1
10.3	1.2	2.1	3.2
14.3	2.9	2.9	4.8
18.3	4.6	6.4	6.5
22.3	2.0	2.3	2.8
26.3	1.4	1.7	1.8
30.3	0.7	0.8	1.3

Field/time after application (h)	MITC air concentration ($\mu\text{g}/\text{m}^3$)		
	200 cm	100 cm	10cm
Mean:	2.5	3.15	3.9

Estimation of respiratory exposure from MITC air concentrations:

Respiratory exposure was calculated by multiplying the average MITC air concentration by average values of lung ventilation in adults.

Respiratory exposure was estimated for a person under light (500kg m/min) or heavy (800kg m/min) conditions of work where the respiration rates were 28.6 and 42.9 liters /min, respectively. Only MITC air concentrations at heights of 100 and 200cm were averaged and used for these calculations. Data for the estimated respiratory exposure a worker might encounter during the re-entry period are shown in Table B.6.15.3.1-3. The maximum dose a person would be exposed to was estimated at 1.4mg per person per day observed 6h following chemigation in field 4. The minimum inhalation exposure of 0.02mg per person per day was found 11h after injection in field 2.

Volatilization losses measured by trapping vaporized MITC indicated that in all treated fields, regardless of application method, average MITC air concentrations were highest in the first 8h following application and decreased thereafter. These observations can be attributed to the fast conversion of metam-sodium to MITC the time for which has been reported to vary between 0.1 and 1 day.

Weather and soil conditions during the first day of sampling also might have contributed to the accelerated MITC loss. Soil temperature was higher during the first day than on the next day of sampling. Wind was also lighter in the first day in comparison to the succeeding day of sampling, indicating less turbulent mixing. Volatilization losses obtained in this study for injected fields, ranged between 1.4 and 0.3 $\mu\text{g}/\text{m}^3$. In the present study, soil temperature averaged 5.6°C in the first three days following fumigation.

The relatively higher MITC air concentrations observed in the chemigated fields (fields 3 and 4) in comparison with the injected fields (fields 1 and 2) may be explained by the larger fraction of chemical placed at the surface by chemigation. The low MITC vapor losses obtained in this study for fumigation using a three-port injection system indicates the environmental superiority of this application method to chemigation. However, further research should compare the effectiveness of each of these application methods on the control of soil-borne pathogens. Although a few nighttime measurements were taken in this study, it was evident that MITC air concentrations during these periods were comparable to those obtained during the daylight hours. These results might be explained by the light wind speeds that prevailed during the nights of measurement causing less mixing in the atmosphere above these fields.

In the present study, it was found that the use of a soil compactor following fumigant injection into the soil had a substantial effect in reducing MITC emissions (Saeed and Rouse, unpublished data). Thus, manipulation of soil surface to reduce MITC loss by volatilization could serve as an approach to environmentally acceptable disease control practices.

It was evident from this study that the 2-day re-entry period after the application of metam-sodium during late fall is an appropriate waiting time to allow MITC emissions to nearly cease. One data point in our results taken at 78h after application indicated that MITC emissions were detectable for at least several days beyond the re-entry period, but levels were low and should not be a problem. However, the relatively high levels of MITC emissions in the first 35h following fumigation may pose a health hazard to humans, domestic animals and wildlife. An effort was made in this study to estimate an average respiratory intake of MITC by a person entering the treated fields during the first 35h after fumigation.

If all MITC in inhaled air were retained by the body, the intake from the highest level of MITC would be 1.37 mg per person per day or 0.019 mg/kg bw/d (70 kg bw) in the chemigated fields. This approach is proposed by the authors of the study and is reported in the table B.6.15.3.1-3 but is not transparent for RMS.

Table B.6.15.3.1-3: Estimated respiratory exposure of a bystander to MITC emitted from metam sodium treated fields during re-entry period.

Field /time after application (h)	Average MITC air concentration (µg/m³)	Range of exposure to MITC as proposed by company	
		mg/person/day light/heavy work	mg/kg bw/day light/heavy work
Injected field 1			
5.3	1.21	0.05/0.30	0.000714/0.00428
11.4	1.45	0.05/0.35	0.000714/0.005
29.5	1.00	0.04/0.25	0.000571/0.00357
Injected field 2			
6	1.1	0.04/0.29	0.000571/0.000414
11.4	0.45	0.02/0.12	0.000285/0.00171
29.5	1.1	0.04/0.27	0.000571/0.00385
Chemigated field 3			
6	1.24	0.04/0.29	0.000571/0.000414
10	0.33	0.05/0.33	0.000714/0.00471
Chemigated field 4			
6.3	5.89	0.21/1.37	0.003/0.0195
10.3	2.17	0.07/0.43	0.001/0.00614
30.3	3.51	0.03/0.20	0.000428/0.00285

Values were averages of mean MITC concentrations measured at 100 and 200 cm heights.

RMS calculated exposure to MITC using 5.89 $\mu\text{g}/\text{m}^3$ concentrations (worst case situation) (respiratory rate of 1.25 m^3/h for a 60 kg bw during 1 hour's exposure period) which gives an exposure of 0.00012 mg/kg bw/d, which represents 3.1% of the AOEL.

Concentration of MITC in air at three heights (10, 100 and 200 cm) above the arable fields were measured following application of Metam-sodium (solution containing 345 kg ai/kg) at an application rate of 480 L/ha corresponding to 166 kg ai/ha. Maximum MITC concentrations in air were recorded 10 cm above ground 6-8 h following application and minimum concentrations of 0.2 $\mu\text{g}/\text{m}^3$ recorded 200 cm above ground 30 – 35 h following application.

Table B.6.15.3.1-4: MITC air concentration measured at 10, 100 and 200 cm above the 4 treated fields.

Field/time after application (h)	MITC air concentration ($\mu\text{g}/\text{m}^3$)			mg/kg bw/day =% of AOEL
	200 cm	100 cm	10cm	
Injected field 1: 166 kg/ha (480L)				
5.3	1.1	1.2	1.3	0.000199= 5%
11.4	1.3	1.4	1.6	
29.4	0.9	1.0	1.1	
78	0.7	0.8	0.9	
Mean:	1.0	1.1	1.22	
Injected field 2: 166 kg/ha (480L)				
4	0.4	0.9	7.0	0.000924 = 23%
6	0.9	1.3	7.4	
11	0.4	0.5	5.9	
27.5	0.3	1.0	1.0	
29.5	0.5	1.6	1.6	
34.5	0.2	0.4	0.4	
Mean:	0.45	0.95	3.88	
Chemigated field 3: 166 kg/ha (480L) diluted in 169m ³ water/ha				
3	1.6	2.0	3.5	0.0014= 35%
6	0.9	1.3	2.8	
8.4	2.0	3.0	11.2	
10	1.2	1.3	4.0	

Field/time after application (h)	MITC air concentration ($\mu\text{g}/\text{m}^3$)			mg/kg bw/day = % of AOEL
	200 cm	100 cm	10cm	
14	0.7	0.8	1.7	
Mean:	1.28	1.7	4.64	
Chemigated field 4: 166 kg/ha (480L) diluted in 169m ³ water/ha				
6.3	4.6	5.9	7.1	0.00088 = 22 %
10.3	1.2	2.1	3.2	
14.3	2.9	2.9	4.8	
18.3	4.6	6.4	6.5	
22.3	2.0	2.3	2.8	
26.3	1.4	1.7	1.8	
30.3	0.7	0.8	1.3	
Mean:	2.5	3.15	3.9	

RMS estimated exposure at the highest doses levels only: exposure to MITC ranged between 0.0014 and 0.00088 mg/person/day (**35**% and **22**% of AOEL).

The result highlighted in bold in the table B.6.15.3.1-4 (6.4 $\mu\text{g}/\text{m}^3$) could be used as the relevant value for worker exposure assessment as well.

- Measured and computed concentrations of methyl isothiocyanate in the air around fumigated fields (Van den Berg, F., 1993)

Material and methods:

Comparison of computer simulation and measured figures:

Behaviour of Metam sodium and MITC in soil as well as the emission of MITC in the air was described by a computer simulation model and results compared with measured figures after soil injection application of Metam sodium into the soil of two selected fields in the Netherlands (field A near Valthermond and field B near Eeserveen).

For both fields, Metam sodium was applied at an application rate of 300 L/ha (510 g Metam sodium/L) and injected at a depth of approx. 18 cm using a horizontal blade injector with spray nozzles below the blades and subsequent compression of the soil with a roller. The treated area comprised 5.9 ha for both fields. The two soils (A/B) had pH values of 4.3 (A) and 5.2 (B), the organic matter content was 13.8 % (A) and 6.2 % (B), the mineral fraction < 2 μm was 2.5 % (A) and 2.2 % (B), and the mineral fraction ranging from 2 – 50 μm was 7.2 % (A) and 6.2 % (B).

During the first few weeks after soil injection the average temperatures in the plough layer of fields A and B were 12 °C and 11 °C, respectively. The concentration of MITC in the air around the two fields was measured four to five times in a period of 7 – 9 days following soil injection of Metam sodium. Sampling of MITC was accomplished by charcoal tubes and air samples were taken in a height of 1.5 m above the soil on each sampling day. Samples were collected at different sampling points with one to two samples taken upwind of the treated field and four to six samples taken at sites downwind of the treated field. Samples were stored under cool and dark conditions prior to extraction with acetone and analysis by GC with NP (nitrogen-phosphorus) detection. The LOD was given with 2.0 $\mu\text{g}/\text{m}^3$ for the measurements on field A (performed 1986) and 1.0 $\mu\text{g}/\text{m}^3$ for the measurements on field B (performed 1987). The concentration patterns of MITC in the air around the treated field were calculated with the PAL (point-area-line) model which is based on the Gaussian plume concept where it is assumed that dispersion from point, area and line sources results in Gaussian distributions in both the lateral and vertical directions through the plume dispersing from that source. Input data for this model comprise field size (km) and orientation, source strength/rate of emission ($\text{g}/\text{m}^2/\text{sec.}$) and source height (m). Hourly values of the MITC emission rate into the air were taken as the source strength and the source height was set to 0 m. In the PAL model the downwind concentration in the air calculated is proportional to the source strength. Further data required by the PAL model are wind speed, wind direction, atmospheric stability and mixing height.

Findings:

The patterns of MITC concentrations measured and calculated at different distances from the source and at different times are shown in table B.6.15.3.1-5:

During the first 3 days after soil injection of Metam sodium and also thereafter the computed data overestimated the actual measured MITC concentration in the air both at different time points and at different distances from the treated fields. The differences between computed and measured MITC concentrations in the air at different distances and time points were due to factors such as differences in the wind speed, wind direction, transformation in soil and emission rates from soil, stability condition of the atmosphere above and around

fumigated fields as well as influence of neighbouring fields and wind obstacles such as houses and trees in real scenarios.

Table B.6.15.3.1-5: Computed and measured concentrations of MITC in the air at downwind of fields A and B at a height of 1.5 m after soil injection application of Metam sodium at a rate of 300 L/h.

Time after injection (days)	Emission rates ($\mu\text{g}/\text{m}^2/\text{sec}$)	Downwind distance (m)	Concentration in air ($\mu\text{g}/\text{m}^3$)	
			computed	measured
Field A				
1.9	0.80	0	4.7	< 2.0
1.9	0.80	51	2.4	< 2.0
1.9	0.80	192	0.8	3.1
2.9	1.30	0	3.9	2.7
2.9	1.30	55	2.0	2.6
5.0	1.10	0	14.0	2.1
5.0	1.10	150	8.2	2.9
6.9	0.79	0	1.8	< 2.0
6.9	0.79	51	1.0	< 2.0
6.9	0.79	99	0.7	< 2.0

Time after injection (days)	Emission rates ($\mu\text{g}/\text{m}^2/\text{sec}$)	Downwind distance (m)	Concentration in air ($\mu\text{g}/\text{m}^3$)	
			computed	measured
Field B				
1.1	0.03	0	0.1	1.8
1.1	0.03	50	0.1	3.1
1.1	0.03	90	0.1	1.1
1.1	0.03	130	0.0	1.0
1.1	0.03	170	0.0	< 1.0
3.1	0.53	0	2.1	< 1.0
3.1	0.53	45	0.9	< 1.0
3.1	0.53	125	0.3	< 1.0
5.2	0.95	0	3.9	1.6
5.2	0.95	40	3.2	1.2
5.2	0.95	74	2.5	< 1.0
5.2	0.95	111	2.1	< 1.0
5.2	0.95	178	1.6	< 1.0
6.9	0.92	36	3.6	1.3
9.1	0.77	0	3.0	< 1.0
9.1	0.77	47	1.4	< 1.0
9.1	0.77	82	1.0	< 1.0
9.1	0.77	130	0.6	< 1.0
9.1	0.77	214	0.4	< 1.0

Both the computed as well as the measured MITC concentrations in the air demonstrated that a bystander walking at the downwind side of a fumigated field (distance 0 – 214 m) approx. 1 to 9 days after treatment is exposed to MITC concentrations amounts to $14 \mu\text{g}/\text{m}^3$. Taking into consideration an exposure time of 10 min for a walk along a treated field and a breathing rate of $0.021 \text{ m}^3/\text{min}$ (corresponding to a moderate respiration rate for medium exercise) an exposure of 0.00294 mg/person or 0.000042 mg/kg bw (based on a body weight of 70 kg/person) can be calculated.

RMS calculated an exposure during 1 hour, based on the $14 \mu\text{g}/\text{m}^3$ (using the maximal computed MITC level at 0 m from the field). This gives an exposure of $17.5 \mu\text{g}/\text{h}$ corresponding to 0.00029 mg/kg bw/hour representing 7.3 % of AOEL (respiration rate: $1.25 \text{ m}^3/\text{h}$; 1 hour; 60 kg bw).

RMS considered the published Van Den Berg study less robust.

- Study of Schepel and Dijksterhuis, 2003, detailed in point B.6.15.4.2.1:

Bystanders might also be present during breaking of the seal after soil fumigation with Metam-sodium or when the plastic film is opened to prepare the soil for planting. In such a case, bystanders might be exposed to remainders of the volatile compound MITC, which is released from the soil due to the breaking of the seal or opening of plastic film coverage. The MITC concentrations in air after breaking of seal or opening/removing of a plastic film coverage before planting can be estimated from the measurements at the edge of the field during and after breaking the seal (experimental protocol detailed under point B.6.15.4.2.1). The maximum air concentration of $8.884 \mu\text{g}/\text{m}^3$ was found 50 m downwind in the time period 0-2 h after breaking the seal 14 days after fumigation with Metam sodium at an application rate of 300 L/ha. Taking into consideration 10 min for a walk beside the edge of the field this would result in an exposure of $1.866 \mu\text{g}/\text{person}$ based on a breathing rate of $0.021 \text{ m}^3/\text{min}$ (corresponding to a moderate respiration rate for medium exercise) corresponding to an exposure of $0.027 \mu\text{g}/\text{kg bw}$ (considering a bodyweight of 70 kg).

RMS calculated an exposure during 1 hour, based on the $8.88 \mu\text{g}/\text{m}^3$: this gives an exposure of $11.1 \mu\text{g}/\text{h}$ corresponding to **0.000185** mg/kg bw/hour representing **4.6** % of AOEL (respiration rate: $1.25 \text{ m}^3/\text{h}$; 1 hour; **60** kg bw).

MITC in the surroundings of the fields:

No MITC was detected in the air surrounding the fields 1 and 2 with one exception; for field 2, which was cultivated 15 days after fumigation and at < 50 m distance to the field a maximum concentration of MITC in air of $0.67 \mu\text{g}/\text{m}^3$ was determined.

In the surroundings of field 3, MITC was found in the air, when cultivation took place 14 days after fumigation. The downwind concentration of MITC in air was $8.88 \mu\text{g}/\text{m}^3$ at 50 m during the first 2 h, but a rapid decrease to $0.63 \mu\text{g}/\text{m}^3$ was noted 6 to 10 h after the cultivation. At 100 m MITC concentration in the air was $4.70 \mu\text{g}/\text{m}^3$ initially, but decreasing to $0.45 \mu\text{g}/\text{m}^3$ after 6 to 10 h after cultivation. When cultivation was done 33 days after fumigation, no MITC was detected in the surroundings (Table B.6.15.4.1-6).

Table B.6.15.3.1-6: MITC concentrations in air surrounding the fields cultivated after fumigation

Field n°	Time (h) after cultivation			
Field 1	0-2 h	2-4 h	4-8 h	24-28 h
day 17	<LOQ	not analysed	not analysed	not analysed
day 21	<LOQ			
Field 2	0-2 h	1-3 h	6-10 h	24-28 h
day 14	<LOQ	<LOQ except left	<LOQ	<LOQ
50 m:		$0.67 \mu\text{g}/\text{m}^3$		
day 21	<LOQ	<LOQ	<LOQ	<LOQ
Field 3	0-2 h	2-4 h	6-10 h	24-28 h
day 14	<LOQ except downwind	<LOQ except downwind:	<LOQ except downwind:	<LOQ
50 m:	$8.88 \mu\text{g}/\text{m}^3$ = 0.000185 mg/kg bw/1h (4.6 % of AOEL)	$2.60 \mu\text{g}/\text{m}^3$	$0.63 \mu\text{g}/\text{m}^3$	
100 m:	$4.7 \mu\text{g}/\text{m}^3$	$1.25 \mu\text{g}/\text{m}^3$	$0.45 \mu\text{g}/\text{m}^3$	
day 33	<LOQ	<LOQ	<LOQ	<LOQ

LOQ = Limit of quantification = $0.01 \text{ mg}/\text{l}$ MITC in extract

- In the investigation of den Tonkelaar and Heidema (details are reported under point B.6.15.4.2.2):

Material and methods of this study are detailed under point B.6.15.4.2.2. Some data from this study are useful for estimation of bystander exposure after metam sodium was applied in a greenhouse.

Bystanders walking along the greenhouse might be exposed to MITC, which is released from the greenhouse into the environment during soil injection application (see Table B.6.15.3.1-7).

The maximum MITC concentration calculated and measured outside of the greenhouse were found at the lee side and amounted to 270 and $182 \mu\text{g}/\text{m}^3$. Taking into consideration an exposure time of 1 hour for a walk along a greenhouse at the lee side and a breathing rate of **$1.25 \text{ m}^3/\text{h}$** (moderate respiration rate for medium exercise) an

exposure to MITC 270 and 182 $\mu\text{g}/\text{m}^3$ corresponds to 5.625 $\mu\text{g}/\text{kg bw/h}$ and 3.792 $\mu\text{g}/\text{kg bw/h}$ (bw 60 kg/person). An exposure to 0.005625 mg/kg bw/h and 0.003792 mg/kg bw/h corresponds to 141% and 95% of the revised AOEL.

At a distance of 20 m downwind of the greenhouse the bystander exposure figures are 4.479 $\mu\text{g}/\text{kg bw/h}$ and 3.708 $\mu\text{g}/\text{kg bw/h}$ corresponding to 112 % and 93 % of the revised AOEL.

At a distance of 50 m and 100 m downwind of the greenhouse, calculated MITC concentrations were highest during the first two days after application and maximum values of about 60 $\mu\text{g}/\text{m}^3$ (0.00125 mg/kg bw/h; 31% of AOEL) and 25 $\mu\text{g}/\text{m}^3$ (0.000521 mg/kg bw/h; 13% of AOEL) were demonstrated. These results were thus, by a factor of 5 – 10 lower on comparison with highest computed concentrations at the lee side of the greenhouse.

Table B.6.15.3.1-7: Bystander exposure estimates to MITC at the lee side and at a distance of 20-100 m downwind of the greenhouse during the first 2 days after soil injection application of Metam sodium at a rate of 1,950 L/ha.

Method	MITC ($\mu\text{g}/\text{m}^3$)	Exposure [#] ($\mu\text{g}/\text{kg bw/hour}$)	% of the revised AOEL
Lee Side			
Calculated	270	5.625	141
Measured	182	3.795	95
20 m Downwind			
Calculated	215	4.479	112
Measured	178	3.708	93
50 m downwind			
	60	1.25	31
100 m downwind			
	25	0.521	13

[#]: exposure duration of 60 minutes assumed; respiration rate 1.25 m^3/h ; bodyweight: 60 kg/person

However, RMS considered that the relevance of this trial was limited, as soil injection in greenhouse was not the supported use in the EU-dossier. A new greenhouse study was available during re-submission, where the product was applied by drip-irrigation (see above in B.6.15.2.4).

- Measured concentrations of methyl isothiocyanate in the air around a fumigated greenhouse after drip-irrigation by Metam (Thouvenin, 2008).

(see table B.6.15.3.1-8)

In the small-scale greenhouse study, a 1% dilution of Nemasol 510 g/L was applied on a surface of 76.5 m^2 (the actually treated surface in a small greenhouse of approximately 6-7 are) at the dose of 1200 L/ha by means of drip-irrigation.

The exposure to the main degradation product MITC was investigated. This included the risk assessment of the operator during loading and application on d1, of the worker on d18, of a bystander present in the vicinity of the greenhouse during 60', and of a resident, present during 24h at remote distances from the treated greenhouse.

Bystander and resident exposure was assessed, taking into account the MITC level measured on static air samplers in a period where outdoor peak-levels were detected (2-14h), in a worst-case scenario.

Table B.6.15.3.1-8: Total exposure values of MITC to bystanders and residents during and after application of Metam in greenhouse, in relation to the proposed AOEL (Thouvenin, 2008).

	bystander (d1)		resident
	1h	1h	24h-day
used value :	mean	max.	max.
concentration (mg/m^3)	0.20462	0.5058	0.00752
sampling time (min)	60	60	1440
inhaled volume (m^3)	1.25	1.25	30
exposure (mg)	0.255775	0.63225	0.22554
exposure ($\text{mg}/\text{kg b.w.}$)	0.0043	0.01054	0.00376
% AOEL, no RPE	107	263	94

Values for the conditions of the study and for a full working day (8h exposure of operator or worker). For the bystander, presence during 60' in the vicinity of the greenhouse is expected; a resident, living at a distance >120m from the greenhouse may be exposed for 24h.
Parameters used for calculations: respiration rate of 1.25 m³/h and a default body weight of 60 kg;
*: Respiratory protective equipment: mask with organic vapour filter (2% penetration); -: RPE not applicable;
AOEL (MITC)= 0.004 mg/kg b.w./d

The exposure of a **bystander**, present in the vicinity (1-12 m) of a treated greenhouse during 60' in a period spanning 2-12h post-treatment, could be considered at the limit of safety, as the AOEL was exceeded by 107% if average MITC levels were considered over the different locations around the tunnel.
If the maximal value was considered (at one location at 10h post-treatment), then the bystander exposure was unsafe, as the AOEL was exceeded by 252%.

B.6.15.4 Summary of estimation of bystander (and resident) exposure (Annex IIIA 7.2.2)

Open point: 2.21 MSs to discuss bystander exposure assessment to metam and MITC.
[See reporting table 2(67)]

The bystander exposure to MITC (field studies only) may be summarised as follows:

The values are taking into account a body weight of 60 kg, a breathing rate of 1.25 m³/h, and the presence of the bystander during one hour (as agreed in the expert meeting).

(i) In the Schepel and Dijksterhuis study, the MITC level on d14 (0-2h after breaking the seal) was 8.88 µg/m³, or 4.6% of the AOEL at 50 m wind downwards (1h exposure). No values at 1-10 m were available but it is speculative whether the MITC-level would be a 100× greater, thereby exceeding the AOEL. Actually, the highest MITC level in the breathing zone of the operator during this seal breaking was 4.62 µg/m³ (which would be equivalent to about 2.4% of the AOEL for a 1h stay of a bystander at this place). Therefore, it is not very plausible that the exposure would be an order of magnitude higher at the edge (1-10m) of a cultivated field. Anyway, the presence of a non-protected bystander during or just after application (soil injection), or during the sheet removal (1-2h) on d14 should be avoidable in a professional context.

(ii) The measurement of MITC level in the most recent field study (Links, 2006), demonstrated that the 1h exposure of an unprotected bystander at 100 m, would consume about 18.7% of the AOEL. On average, the exposure of a bystander present in any direction during one hour amounted to about 2.7% of the AOEL.

(iii) When the highest ambient MITC level was taken (10h post-application of metam in a tunnel, Thouvenin, 2008), the AOEL was exceeded by a factor of about 2.5. It was noted that this was a highly conservative approach. When an multi-directional average was considered, the exposure of a bystander, staying 1h in the neighbourhood of the treated tunnel would be approximately at the level of the AOEL (107%).

It was of note that not all risk-mitigating measures were taken during this trial. For example, the entrances of the greenhouse/tunnel were not closed during and after the treatment. Therefore, it is quite probable that the exposure of the bystander in the neighbourhood of the treated greenhouse at 2-14h after the start of treatment could be reduced, if MITC would be contained into the greenhouse during that lapse of time.

Bystander exposure is summarised in the table B.6.15.4-1:

In conclusion, taking into account all scenarios where an acceptable exposure of the operator to MITC was demonstrated, no undue risk for a bystander was anticipated when metam was applied in full field.

When metam was applied in a greenhouse or a tunnel RMS considered that a safe use was questionable for a bystander, being present in the immediate vicinity of a treated and open greenhouse.

Taking into the rapid degradation of Metam-sodium, and the particular way of application in the soil (soil injection or drip irrigation), the issue of bystander exposure to Metam itself is considered irrelevant.

Table B.6.15.4-1: Measurements of bystander (and resident) exposure to MITC :

Exposure to MITC	Bystander exposure to MITC		Experimental conditions
	µg/m³	in mg/kg b.w./d % AOEL*	
Soil injection- field conditions:			
Links et al, 2006	36	0.00075 18.7%	Metam sodium, 1 field of 5 Ha; 1500L metam sodium applied Rumpststad shear injector; distance 100 m; during application
Saeed et al, 2000	5.89	0.00012 3.1 %	Metam sodium, 510 g/L, soil injection/chemigation; MITC measured in air 6 h after application
Van Den Berg, 1993	14	0.00029 7.3 %	Metam sodium, soil injection, computer simulation, 1.1-9 d after application; 1.5 m height
Soil injection: breaking of seal or opening/removing of seal			
Schepel and Dijksterhuis, 2003	8.88	0.000185 4.6%	Seal was broken 14 d after soil treatment; 0-2 h measure after breaking; metam sodium application at 300L/ha
Drip-irrigation, greenhouse			
Thouvenin, 2008			
Bystander		0.01054 ⁽¹⁾ [0.00426 ⁽²⁾] 264% ⁽¹⁾ [107 ⁽²⁾]	Metam sodium (1% dilution, 1200 L/ha, drip-irrigation in tunnel, MITC measured in air at about 1.5 m height, 10 h after application, at 1-12m from the (open) entrance of the greenhouse
Resident		0.00016 3.9	Metam sodium (1% dilution, 1200 L/ha, drip-irrigation in tunnel, MITC measured in air at about 1.5 m height, 10 h after application, at 140-220m from the (open) entrance of the tunnel

calculations based upon the presence during 1h (bystander) or 24h (resident); b.w.= 60kg; respiration rate 1.25 m³/h; maximal value ⁽¹⁾ or multi-direction average ⁽²⁾ used; comparison with the revised AOEL (0.004 mg/kg b.w.d)

B.6.15.5 Estimation of worker exposure (Annex IIIA 7.2.3.1)

B.6.15.5.1 Estimation of worker exposure to metam sodium (Annex IIIA 7.2.3.1)

Metam-sodium is used as soil fumigant and very rapidly releases the active principle MITC on contact with soil during application by soil injection or drip irrigation, respectively. Due to the rapid degradation of Metam-sodium to MITC, no exposure of workers towards Metam-sodium is considered and no worker risk assessment is required.

B.6.15.5.2. Estimation of worker exposure to MITC (Annex IIIA 7.2.3.1)

B.6.15.5.2.1 Estimation of worker exposure towards MITC after field application of Metam sodium (Annex IIIA 7.2.3.1)

For the intended application of Metam-sodium as soil fumigant worker re-entry tasks cannot be estimated with the existing BBA model for worker re-entry (Hoernicke, E. et al (1998)) because exposure is not related to potential exposure to foliage.

At the time when Metam-sodium is applied and at the time when workers will enter the treated area (about 14 days after treatment to take a soil sample for cress test or to break the seal and/or open plastic film to prepare for planting) no plants are available in the field and worker exposure will be exposed towards the volatile degradation product MITC, which is the active compound whereas Metam-Sodium is the pre-cursor and not of relevance anymore, when the worker re-entry took place. Therefore, MITC exposure is considered for the worker re-entry tasks only.

A study from Schepel and Dijksterhuis (2003) is available where measurements that enable the estimation of potential worker exposure are performed. This study is summarised in the following.

- Operator and bystander inhalatory exposure to MITC during and after breaking of the seal after soil fumigation with Metam-sodium (Schepel and Dijksterhuis, 2003)Material and methods:

Metam-sodium (Luxan Monam (Metam-sodium 510 g/L; batch: G070009514 (batch 1); G0A010564 (batch 2) purity: 508 g/l (batch 1); 501 g/l (batch 2) was applied onto three sandy soil fields in the Northeast of the Netherlands with a rate of 300 l/ha of a 510 g/l Metam-sodium solution. In every field of the study, two separate parts (2 ha each) were cultivated at different time points. Cultivation means breaking of the seal to let the remaining MITC dissipate from the soil. The breaking of the seal took place 14 to 17 days after fumigation (first time) and 21 to 33 days after fumigation (second time). All the investigations were done in the autumn (mid of October until mid of December) of 2001.

During the breaking of the seal (from the beginning until the end of cultivation of each test plot), air at the level of the tractor cabin and air from the breathing zone of the driver was sampled and was investigated for the amount of MITC. Furthermore, MITC concentration in the surroundings of the fields was measured by means of sampling pumps set up at 50 and 100 m downwind and at right angles to the wind direction. The pumps were installed at 1.5 m height. Sampling was performed at different time intervals after the soil breaking; 8 h sampling starting with the beginning of cultivation (subdivided in 2, 2, and 4 h sampling periods) and 4 h sampling starting 24 h after the cultivation.

The MITC exposure on the fields was measured under controlled conditions by means of tunnels with sampling pumps at the inlet and outlet of the air. Sampling was performed during different time intervals after the soil breaking to determine the emission flux for MITC directly at the soil surface.

Soil parameters such as soil temperature, pH, and organic matter content were determined for each treated soil in addition to meteorological parameters such as wind speed, wind direction, air humidity and air temperature, respectively. Soil samples were taken one to two days before the breaking of the seal, in order to determine the MITC residue in the top layer of the soil.

MITC concentrations in air were determined by using air pumps with a flow-through of 40 L/h. Pumps were connected to an SKC adsorbent tube with petroleum based charcoal as the adsorbent material. After termination of sampling adsorbent material was extracted and extract was analysed by GC/NPD.

Blank tests for determination of potential MITC background concentration in air were taken 25 meters from the edge of the field (windward side) for 8 h starting with the beginning of cultivation. Twenty-four hours after cultivation another measurement was made for 4 h. In addition the adsorption capacity of the adsorbent tubes used was investigated for the MITC air measurements and for the MITC emission flux measurements (i.e. breakthrough samples). In addition, a test was conducted to check if a known quantity of applied MITC is still quantifiable after 2 or 4 h of aeration and after sample preparation as for the other samples taken (fortification of SKC tubes – field spikes).

Analytical investigations:

Fortification experiments with SKC tubes (field spikes) demonstrated that average recovery of MITC at a level of 0.01 mg/l was 84 % and at a ten-fold higher level it was 82 %. These results are within the criteria for method validation (SANCO/825/00 rev. 6 “Guidance document on residue analytical methods” dated 20 June 2000).

On the basis of the analytical method used the limit of detection (LOD) was shown to be 0.01 mg/L in the extract. No penetration of MITC to a second SKC tube could be demonstrated and MITC was efficiently adsorbed onto the activated carbon (no breakthrough).

Findings:MITC concentrations in soil:

In untreated fields no MITC was found (controls).

In treated fields MITC was found at levels of about 0.1 up to 14.96 µg/g dried soil, 13 to 31 days after fumigation with Metam-sodium.

MITC concentrations were measured at the breathing zone of driver and in the tractor cabin:

During the first soil cultivation 14-17 days after fumigation, an amount of MITC was detected with an average exposure to MITC of 1.26 µg/m³ in the breathing zone and 1.88 µg/m³ in the cabin (table B.6.15.5.2-1). When the soil cultivation was performed after 3 to 4.5 weeks, MITC was not detected in the driver's breathing zone or in the cabin.

The highest MITC concentration was 4.62 µg/m³ at the breathing zone of a driver who did the cultivation 14 days after fumigation.

Table B.6.15.5.2.1-1: MITC concentrations in the driver's breathing zone and in the tractor cabin.

Location/ fumigation date	Days after fumigation	in the breathing zone ($\mu\text{g}/\text{m}^3$)	mg/kg bw/d % AOEL	in the cabin ($\mu\text{g}/\text{m}^3$)	mg/kg bw/d % AOEL
Field 1/ 15 Oct 2001	17 22	<LOQ <LOQ		0.97 <LOQ	
Field 2/ 25 Oct 2001	14 21	4.62 <LOQ	0.00077 19.25%	3.42 <LOQ	0.00057 14.25%
Field 3 7 Nov 2001	14 33	1.26 <LOQ		1.88 <LOQ	

LOQ = Limit of quantification = 0.01 mg/L MITC in extract

Revised AOEL = 0.004 mg/kg bw/d; working time=8h/d; inhalation rate = 1.25 m³/h; b.w.= 60 kg.Risk assessment worker re-entry:

For risk assessment purposes the measured MITC exposure levels are compared to AOEL of MITC. For this purpose exposure of workers via the inhalation route is considered which is justified as worker re-entry tasks are performed 10 days after fumigation with Metam-sodium at the earliest, with no foliage available and with Metam-sodium being already degraded. Thus, only release of volatile MITC as a consequence of Metam-sodium degradation is to be considered for the following potential worker re-entry tasks:

Table B. 6.15.4.2.1-2:

- Sampling of soil for the cress test (worker re-entry scenario 1):
The sampling of soil will be a worker re-entry task of very short duration (max. 15 minutes) and thus, it does not represent worst case compared to other re-entry scenarios. The worker re-entry assessment is covered by the estimates for the other re-entry tasks (preparation of soil and plastic film opening).
- Preparation of soil (opening/breaking of soil) (worker re-entry scenario 2):
Cultivation of the soil by using tractor mounted equipment is considered with a working time of 8 h. The mean concentrations of MITC in air in breathing zone of the tractor driver and in the cabin were similar with about 4 $\mu\text{g}/\text{m}^3$. This MITC concentration will be used to estimate potential worker re-entry exposure.
- Plastic film opening (preparation of holes to plant e.g. strawberries, melons etc.) (Worker re-entry scenario 3): Preparation for planting by opening the plastic film is considered for a working time of 8 h. For the estimation of potential worker re-entry exposure the highest MITC concentration noted in measurements for the surrounding area will be used (about 8.88 $\mu\text{g}/\text{m}^3$). Considering a 8 h exposure and using this figure is considered to be worst case, because it is the highest MITC concentration found downwind close to the treated area, the figure was only seen within the first 2 h after breaking the seal in one field (at the other two fields no MITC or significantly less MITC was detected and MITC concentration dropped from 8.88 to 2.60 and 0.63 $\mu\text{g}/\text{m}^3$ at the next samplings representing the periods 2-4 h and 4-8 h, respectively after breaking the seal).

The worker re-entry exposure calculations presented in table B.6.15.4.2.1-2 are based on measurements of MITC concentrations in air in the field after soil injection application of Metam sodium at an application rate of 300 L/ha. To calculate the exposure a breathing rate of 1.25 m³/h and a body weight of 60 kg was taken into consideration.

However, in the UK proposal of 60 kg bw and an 8h working day, the figure would be: (1.25 m³/h×8h= 10m³, × 0.00888 mg/m³ = 0.0888 mg/day, ÷60 kg = 0.00148 mg/kg bw/d), thus representing about 37%, (and not 116%) of the AOEL, which is still acceptable. Summary calculations (table B.6.15.5.2.1-2) were performed taking into account a 60 kg b.w. and a work rate of 8h/d.

Table B. 6.15.5.2.1-2:

Worker re-entry exposure for MITC and comparison to AOEL (Schepel and Dijksterhuis, 2003)

Worker re-entry scenario	Working time (h)	concentration ($\mu\text{g}/\text{m}^3$)	Exposure (mg/person/d)	Exposure (mg/kg bw/d)	% AOEL
Sampling of soil for cress test (1)	0.25	scenario 1 covered by estimates for scenario 2 and 3			
Preparation of soil (2)	8	4.62	0.046	0.00077	19%
Plastic film opening (3)	8	8.88	0.088	0.00148	37%

Revised AOEL = 0.004 mg/kg bw/d; working time=8h/d; inhalation rate = 1.25 m³/h; b.w.=60 kg

Taking into account the rapid degradation of Metam-sodium, no substance will remain at re-entry (2-3 weeks after application), therefore the issue of worker exposure to Metam-sodium itself was also considered irrelevant. Conclusion of RMS: the results reported in the table B.6.15.5.2.1-2, show that worker exposure based on measurements of MITC in air in the field gives an exposure below the revised AOEL.

- MITC concentration in air measured at 10, 100 and 200 cm above the treated field (See point B.6.15.3 study of Saeed et al, 2000, detailed under bystander exposure):

From the data reported in the table B.6.15.3.1-4, worker exposure can be estimated. Using the data from the chemigated field, which is a worst-case situation, 6.4 µg/m³ MITC was detected at 100 cm above the soil, 18 hour after treatment of the soil (maximal value).

From this data, worker exposure represents 0.001067 mg/kg bw/d, which is 27% of the AOEL.

- Emission and Spreading of MITC with Soil Decontamination in Glasshouses (Emissie en Verspreiding van Methylisothiocyanat bij Grondontsmetting in Kassen) (den Tonkelaar and Heidema, 1988)

Material and methods:

Metam sodium (Metam-sodium 510 g/L) was applied in a greenhouse by soil injection and the concentration of MITC was measured inside and outside of the greenhouse. The treated area in the greenhouse was 1,230 m² (3.1 m x 33 m) and a total of 240 L of Metam sodium was used corresponding to an application rate of approx. 1,950 L/ha. The height of the greenhouse ranged from 2.3 m at the gutter to 2.5 m at the ridge. Application was carried out by a self-propelled tooth injector at an injection depth of 5 – 10 cm. The treated area in the greenhouse was covered with low-density-poly-ethylene (LDPE) foil of 30-µm thickness within 5 – 10 minutes after injection was finished. Seven days after application the foil seal was broken and completely removed 2 days later. During the test, the concentration of MITC within and near the greenhouse was measured in addition to the temperature under the foil at two different sites within the greenhouse and outside of the greenhouse. Other parameters measured comprised humidity in the greenhouse and wind speed as well as wind direction 2.2 m above the greenhouse. Several hourly air samples were taken to determine the MITC concentration in the airflow on the lee side of the greenhouse and at a distance of 20 m downwind from the greenhouse. For collection of samples, the suction speed in the air was 0.5 L/min. at the lee side and 1.125 L/min at a distance of 20 m from the greenhouse. For the determination of the ventilation rate, the reduction of the concentration of the tracer gas Sulfur hexafluoride (SF₆) was measured inside and outside of the greenhouse. The ventilation rate was calculated by means of an exponential function where besides the position of the windows (open, half-open, shut) fluctuations in the wind speed have the largest influence. A further objective of the study was to develop a model for the calculation of the emission of MITC from the greenhouse into the environment and its distribution in the nearby environment. The concentration of MITC inside the greenhouse was measured by an Infrared spectrometric method. The IR-spectrometer was calibrated with known concentrations of MITC in the air and linearity of the method was demonstrated. For the determination of the MITC, concentration outside of the greenhouse a GC method with ECD (electron capture) detection was used. MITC samples were adsorbed onto SKC charcoal tubes, which were eluted with acetone. The eluate was stored at – 30 °C until gas chromatographic analysis.

Ventilation rate measurements:

In the experiments carried out with the tracer gas SF₆ six times during a period of three days it could be shown that the contribution of leakage to the reduction of the concentration of the gas in the greenhouse was 1 ± 15 % per hour, which means that the hourly ventilation rate corresponded to the wind speed (expressed in m/sec.). Since the average wind speed was about 4 m/sec. a ventilation rate of 100 times/d was derived thereof.

After single introduction of the tracer, gas into the glasshouse the reduction of the concentration of the tracer gas shows that dilution occurs in an exponential manner and demonstrated linearity for the duration of the experiment. Since only a graph was presented in the study report approximate figures are given in the table below.

Table B.6.15.5.2.1-3

Reduction of SF₆ concentration within the greenhouse for determination of the ventilation rate

Time (min)	0	10	20	30	40	50	60	70	80
SF ₆ (mg/m ³) ¹	26	18.7	14	9	6.4	4.4	2.7	2	1.5

¹: only approximate concentrations given

Findings:

Decline of MITC concentrations in the greenhouse:

From the MITC concentration measurements performed in the greenhouse (table B.6.15.5.2.1-4a), it could be seen that there was a decline from initially about 65 mg/m³ MITC about 12 h after application to approximately 4.7 mg/m³ MITC at 120 h after application. In addition, the ventilation/emission from the greenhouse to the outside was demonstrated to be not the rate-determining step. With SF₆ one hour after introduction of the tracer

gas about 10 % of the initial and maximum concentration was found while for MITC this took about 4 – 5 days under the conditions of the application taking into account the application rate, the ventilation rate as well as temperature and humidity. The concentration of MITC in the greenhouse and the corresponding ventilation to the outside of the greenhouse was therefore determined by other factors such as transformation rate from Metam sodium, diffusion behavior in the soil as well as velocity and degree of diffusion through the plastic foil. The development of the concentrations of MITC within the greenhouse with time is shown in the table below. Since figures were taken from a graph, approximate concentration values could be indicated which, however, are suitable for a good estimate of the course of MITC concentrations at different times after soil injection application was carried out.

Table B.6.15.5.2.1-4a: Course of MITC concentrations in the greenhouse during a period of 5 days after soil injection application of Metam sodium at a rate of 1,950 L/ha

Time (h)	12	24	36	40	48	54	60	72	78	84	96	108	120
MITC(mg/m ³) ¹	65	52	40	49.5	33.7	51.6	42	22.6	27.9	17.9	8.4	8.4	4.7

¹: only approximate concentrations given

As is shown in table B.6.15.5.2.1-4b, MITC concentration declined to about 2.6 mg/m³ after 6 ½ days with another peak concentration of 4.7 mg/m³ on day 7, which is due to partial removal of the protective plastic tarp.

Table B.6.15.5.2.1-4b: Course of MITC concentrations in the greenhouse from day 5 until day 9 after soil injection application of Metam sodium at a rate of 1,950 L/ha

Time (h)	120	132	144	156	168	180	192	216
MITC (mg/m ³) ¹	4.7	2.6	1.6	2.6	4.7	1.6	1	1

¹: only approximate concentrations given

In general, no re-entry into the greenhouse is required during the first 10 days after application of Metam sodium. In certain instances access to the greenhouse might become necessary during the first 5 – 7 days after application by soil injection was carried out and workers etc. will thus, be exposed to MITC which is present in the air of the treated greenhouse.

Taking a respiration rate of 1.25 m³/h into account, a body weight of 60 kg/person, and an exposure time of 8 h, exposure will amount to 10.83 mg/kg bw/d 12 h after application (65 mg/m³) and to 0.783 mg/kg bw/d 5 days after application (4.7 mg/m³). (see table B.6.15.5.2.1-5).

Table B.6.15.5.2.1-5: MITC exposure estimates in the greenhouse and comparison with revised AOEL during the first 5-7 days after soil injection application of Metam sodium at a rate of 1,950 L/ha.

Time post-treatment (h)	MITC (mg/m ³)	Exposure (mg/kg bw/day)	fold revised AOEL
Exposure for a whole working day (8h):			
Without RPE :			
12 h	65	10.83	× 2708
24 h	52	8.67	× 2167
48 h	33.7	5.62	× 1404
72 h	22.6	3.77	× 942
96 h	8.4	1.40	× 350
120 / 168 h	4.7	0.78	× 196
With RPE :			
12 h	65	0.217	× 41
120 / 168 h (day 7)	4.7	0.016	× 3.9
Exposure reduced to 1 hour:			
12 h	65	1.35	× 339
With RPE:			
120/168 h	4.7	0.027	× 6.8
With RPE:			
120/168 h	4.7	0.098	× 24
With RPE:			
120/168 h	4.7	0.00196	× 0.49

calculations performed for a working time=8h/d, then recalculated for 1h/d (occasional re-entry);

Revised AOEL = 0.004 mg/kg bw/d; inhalation rate = 1.25 m³/h; b.w.=60 kg; RPE: A1P2 filter mask (2 % penetration)

Re-entry into the greenhouse is neither intended nor required until removal of the plastic tarp from the treated soil.

In cases, where unscheduled re-entry is required and task will be of short duration, i.e. 1 h, respiratory protection has to be worn, i.e. a A1P2 type filter mask, which reduces exposure by a factor of 50 resulting in 2 % of the exposure encountered without respiratory protective equipment. Taking into account the figures re-entry 12 h after application would result in 10.83 mg/kg bw/d x 0.02 (2 % penetration through A1P2 type filter mask) = 0.217 mg/kg bw/d which is much higher (41 times) than the revised AOEL.

Furthermore, a peak concentration of 4.7 mg/m³ was measured on day 7 when the plastic foil was partially removed from the treated area in the greenhouse. Without any protection and taking into account, a working time of 8 h, worker exposure amounts to 0.78 mg/kg bw/d. This shows that during the task "removal of plastic tarp" respiratory protection is not sufficient (0.0117 mg/kg bw/d = 3.9 times the revised AOEL) (see Table B.6.15.4.1-6).

Based on the exposure figures measured 7 days after soil injection application of Metam sodium, a 1 hr exposure associated with re-entry (0.00196 mg/kg b.w./d) into the greenhouse is below the revised AOEL (49%) only if RPE is worn during removal of the plastic tarp.

If the task is reduced to 1 hour, but performed 12 h after application, worker exposure reaches 1.16 mg/kg bw/d but RPE does not reduce sufficiently the exposure.

Conclusion: a re-entry time of at least 7d is necessary as well as respiratory protection conferring at least a 50× protection.

Concentrations of MITC outside of the greenhouse:

The outdoor concentrations of MITC were measured and calculated at the lee side and at a distance of 20 m downwind of the greenhouse. Further calculations were performed at distances of 50 and 100 m downwind of the greenhouse.

The results of the measurements and calculations performed for MITC concentrations in the air at the lee side and at a distance of 20 m downwind of the greenhouse one and two days after application are shown in table B.6.15.4.2.1-6. The measured figures were always lower than the calculated MITC concentrations. In addition, the highest MITC concentrations were directly behind the greenhouse both after measuring and calculating MITC concentrations in the outdoor air. After 2 days, concentrations of MITC in the air were declining and figures were well below 100 µg/m³. The course of concentrations of MITC in the outside air was the same as in the inside air since a higher wind speed results in higher emission rates in addition to a higher dilution. Thus, these two effects do compensate each other.

Table B.6.15.5.2.1-6:

Computed and calculated MITC air concentrations outside the greenhouse after soil injection application of Metam sodium at a rate of 1,950 L/ha. Worker exposure is compared with the revised AOEL

Lee side (no RPE)					Lee side (in the presence of RPE)			
	comp. mg/m ³	mg/kg bw/ d % AOEL	meas. mg/m ³	mg/kg bw/ d % AOEL	comp. mg/m ³	mg/kg bw/ d % AOEL	meas. mg/m ³	mg/kg bw/ d % AOEL
Day 1	0.270	0.045 1125	0.081	0.0135 338	0.270	0.0009 22.50	0.081	0.0027 6.75
	0.250	0.0417 1042	0.182	0.0303 758	0.250	0.00083 20.83	0.182	0.00061 15.17
	0.220	0.0367 917	0.034	0.0057 142	0.220	0.00073 18.33	0.034	0.00011 2.83
Day 2	0.195	0.0325 813	0.070	0.0117 292	0.195	0.00065 16.25	0.070	0.00023 5.83
	0.195	0.0325 813	0.082	0.0137 342	0.195	0.00065 16.25	0.082	0.00027 6.83
	0.190	0.0317 667	0.025	0.0042 104	0.190	0.00063 15.83	0.025	0.00008 2.08
	0.160	0.0267 667	0.041	0.0068 171	0.160	0.00053 13.33	0.041	0.00014 3.42
	0.165	0.0275 688	0.030	0.0050 125	0.165	0.00055 13.75	0.030	0.00010 2.5
	0.165	0.0275 688	0.048	0.0080 200	0.165	0.00055 13.75	0.048	0.00016 4.00
	0.160	0.0267 667	0.032	0.0053 133	0.160	0.00053 13.33	0.032	0.00011 2.67
Downwind, 20 m distance (no RPE)					Downwind, 20 m distance (in the presence of RPE)			
	comp.	mg/kg bw/ d % AOEL	meas.	mg/kg bw/ d % AOEL	comp.	mg/kg bw/ d % AOEL	meas.	mg/kg bw/ d % AOEL
Day 1	0.215	0.0358 896	0	0	0.215	0.00072 17.92	0	0
	0.200	0.0333 833	0.058	0.0097 242	0.200	0.00067 16.67	0.058	0.00019 4.83
	0.175	0.0292 729	0.178	0.0297 742	0.175	0.00058 14.58	0.178	0.00059 14.83
Day 2	0.155	0.0258 646	0.065	0.0108 271	0.155	0.00052 12.92	0.065	0.00022 5.42
	0.155	0.0258 646	0.065	0.0108 271	0.155	0.00052 12.92	0.065	0.00022 5.42
	0.150	0.025 625	0.068	0.0113 283	0.150	0.00050 12.50	0.068	0.00023 5.67
	0.125	0.0208 521	0.053	0.0088 221	0.125	0.00042 10.42	0.053	0.00018 4.42
	0.130	0.0217 542	0.018	0.003 75	0.130	0.00043 10.83	0.018	0.00006 1.50
	0.130	0.0217 542	0.005	0.0008 21	0.130	0.00043 10.83	0.005	0.00002 0.42
	0.125	0.0208 521	0.002	0.0003 8.33	0.125	0.00042 10.42	0.002	0.00001 0.17

calculations performed for a working time=8h/d, Revised AOEL = 0.004 mg/kg bw/d; inhalation rate = 1.25 m³/h; b.w.=60 kg; RPE: A1P2 filter mask (2 % penetration); comp.= computed value; meas.= measured value

- Measured concentrations of methyl isothiocyanate in the air around a fumigated greenhouse after drip-irrigation by Metam (Thouvenin, 2008).

In the recent small-scale greenhouse trial, a 1% dilution of Nemasol 510 g/L was applied on a surface of 76.5 m² (the actually treated surface in a small greenhouse of approximately 6-7 are) at the dose of 1200 L/ha by means of drip-irrigation.

The exposure to the main degradation product MITC was investigated. This included the risk assessment of the worker on d18.

The inhalation exposure of the worker was assessed by measuring MITC levels during 30 min in the breathing zone, and the value was extrapolated for a full 8h working day.

Table B.6.15.5.2.1-7: Total exposure values of MITC to the worker after application of Metam in greenhouse, in relation to the revised AOEL (Thouvenin, 2008).

	worker (d18)	
	study	8h-day
concentration (mg/m ³)	0.00071	0.00071
sampling time (min)	30	480
inhaled volume (m ³)	0.625	10
exposure (mg)	0.00044	0.0071
exposure (mg/kg b.w.)	0.0000074	0.000118
% AOEL, no RPE	0.18	2.96
% AOEL, RPE (98%)*	0.0037	0.059

Values for the conditions of the study and for a full working day (8h exposure) of the worker. Parameters used for calculations: respiration rate of 1.25 m³/h and a default body weight of 60 kg;

*: Respiratory protective equipment; mask with organic vapour filter (2% penetration);

AOEL (MITC)= 0.004 mg/kg b.w./d

The exposure of the **worker**, re-entering the greenhouse after 18 days, in order to drill plant holes in the treated bands, was acceptable, even in the absence of RPE (exposure accounting for 2.96% of the AOEL in the absence of RPE, and 0.06% in the presence of RPE).

B.6.15.6 Summary of estimation of worker exposure (Annex IIIA 7.2.2)

(i) In the field:

Worker re-entry exposure calculations based on measurements of MITC concentrations in air are below the AOEL proposed by the RMS.

(ii) In glasshouse (soil injection trial): After soil decontamination in glasshouses, workers should not enter the greenhouse shortly after soil injection application and a waiting period of 120-168 h with a respiratory protection is necessary to be below the AOEL. Concentrations of MITC outside the greenhouse were quite high reaching sometimes 182 µg/m³ and 178 µg/m³ on the lee side and at 20 m downwind 1 day after application, respectively.

The worker, removing the seal and working the soil on 14-17d post-application was exposed to an MITC level as high as 8.8 µg/m³, and the internal dose during an 8h-working period amounted to 1.48 µg/kg b.w./d, corresponding with 37% of the AOEL.

However, RMS considered that the relevance of the first greenhouse study was limited, as soil injection was not the supported use in the EU-dossier. A new greenhouse study was available during re-submission, where the product was applied by drip-irrigation (see above in B.6.15.2.4).

(iii) The inhalatory exposure of the *worker* during hole drilling of the treated soil by drip-irrigation in a greenhouse (d18), represented 0.18% of the AOEL, in the absence of RPE. If the cultivation would be performed with a respirator, then about 0.004% of the AOEL of MITC would be attained.

The extrapolated value for a whole working day is also well below the AOEL, both in the absence (about 3%) and in the presence (0.06%) of RPE.

The worker exposure to MITC may be summarised as follows (table B.6.15.4):

The values are taking into account a body weight of 60 kg, a breathing rate of 1.25 m³/h, and the presence of the worker during 8 hours (as agreed in the expert meeting).

Table B.6.15.6: Estimation of worker exposure to MITC following application of Metam sodium

Experimental conditions	µg/m ³	mg/kg bw/d	% of AOEL	Reference
Soil injection: open field	Seal broken 14-17 days after soil treatment; 0-2 hour measure after breaking; metam sodium application at 300L/ha			Schepel and Dijksterhuis, 2003
Preparation of soil	3.42-4.62	0.00057-0.00077	14-19%	
+RPE			0.3-0.4%	
Plastic film opening	8.88	0.00148	37%	
+RPE			0.74%	Saeed et al., 2000
Volatilization from chemigated field at 100 cm height at 18 h post application	6.4	0.00107	27%	
+RPE			0.54%	
Soil injection: greenhouse ^s	Seal broken 14-17 days after soil treatment; 0-2 hour measure after breaking; metam sodium application at 300L/ha			Den Tonckelaar and Heidema, 1988
Preparation of soil	3.42-4.62	0.00057-0.00077	14-19%	
+RPE			0.3-0.4%	
Plastic film opening	8.88	0.00148	37%	
+RPE			0.74%	Thouvenin, 2008
Drip irrigation: greenhouse	Drip-irrigation in tunnel, MITC 30' measure in the breathing zone while worker was drilling holes in the fumigated soil, on d 18 after application of metam (1% dilution) at 1200L/ha.			
Hole drilling d18 post application	0.71	0.00018	2.96%	
+RPE			0.06%	

• calculations based upon working during 8h; b.w.= 60kg; respiration rate 1.25 m³/h.

• +RPE: respirator with A1P2 filter (2% penetration).

^s: scenario not relevant for worker exposure, as soil injection in greenhouses not supported any more in the EU-dossier.

The exposure of the worker to MITC, on d14 following application of Metam did not give rise to concern in open field (soil-injection) or in a greenhouse (drip-irrigation), neither in the absence nor in the presence of RPE.

B.6.15.7 General summary and conclusions:**Operator, worker and bystander exposure to MITC after application of Metam**

Measurements under realistic open field conditions ; values as % of AOEL of MITC (0.004 mg/kg b.w./d); RPE (operator, worker) : combination filter A1P2, 2% penetration; figures including worst-case values
Operator, bw= 70kg, duration of the loading and application: 7 h/d
Worker, bw= 60kg, duration of the cultivation, seal breaking and/or plant hole drilling: 8 h/d
Bystander, bw= 60kg, presence in the neighbourhood of field or greenhouse at peak-level: 1 h/d

Operator exposure (personal measurements);

-Study 1*, soil injection:	450% of AOEL, w/o RPE	and 9.0% with RPE
-Study 2*, soil fumigation,	229% of AOEL, w/o RPE	and 4.6% with RPE
-Study 3*, soil injection,	239% of AOEL, w/o RPE	and 4.8% with RPE
-Study 4**, drip-irrigation;	145% of AOEL, w/o RPE	and 2.9% with RPE

[*: open field studies, **: newly submitted greenhouse/tunnel study]

Worker exposure ; 14-17 day after soil injection,**0-2 hr after breaking seal, personal measurements:**

-Preparation of soil:	14-19% of AOEL w/o RPE	and 0.3-0.4% with RPE
-Plastic film opening:	37% of AOEL w/o RPE	and 0.74% with RPE
-Volatilisation from field, 18h post application, 1 m height	27% of AOEL w/o RPE	and 0.54% with RPE
-Hole drilling through plastic film, 18 days after greenhouse treatment (drip irrigation); personal measurement	2.96% of AOEL w/o RPE	and 0.06% with RPE

Bystander exposure (no RPE assumed):

-soil injection, distance of 100 m, during application:	18.7% of AOEL
-soil injection, MITC in air 6 hour after application:	3.1% of AOEL
- soil injection, 1.1-9 days after application, 1.5 m height:	7.3% of AOEL
-drip irrigation in greenhouse, 10 h after application, at open side of greenhouse:	107-264% of AOEL

Resident exposure (no RPE assumed):

drip irrigation in greenhouse, 10 h after application, 140-220m distance of greenhouse: 3.9% of AOEL

In conclusion, the exposure to MITC is unacceptable for an operator, in the absence of RPE. In the presence of RPE, the measured exposure was at most 9% of the AOEL.

For the worker, the exposure was below the AOEL, but as there was only a MOS of a factor of 2.7 without protection, the wearing of RPE is also highly recommended.

The exposure of a bystander, staying 1h in the neighbourhood of a freshly fumigated **field** is also below the AOEL, amounting to 19% of the AOEL in the worst case. However, at 10h post-application in a **greenhouse** (in fact an open tunnel), the ambient MITC level indicates that a bystander, staying 1 hour at the lee side of the open tunnel would be exposed at about 2.6× the AOEL. A less conservative approach, where average MITC levels were calculated from different sides of the greenhouse, or a time-weighted average for the period 2-14h post-treatment only at the open side of the tunnel, indicates that the exposure would be at the level of the AOEL.

A resident, living at a distance of >140m from the treated greenhouse, would be exposed to about 4% of the AOEL, taking into account a presence during 24h when peak-levels of MITC were generated.

Overall, the application of Metam *undiluted* in **open field** by soil injection (including spot-application in grapes) is considered to be safe, if appropriate RPE is present.

The application in a **greenhouse** was not demonstrated to be safe in the case of soil injection.

The use of drip irrigation (Metam applied at 1% dilution in a tunnel) was safe for the operator and the worker, but the existing study did not exclude a risk for the bystander in a worst-case scenario.

However, it is proposed to handle the potential risk of the bystander by risk-mitigating measures (sufficient warning labels and installation of a safety perimeter around freshly treated *closed* greenhouses). It is stressed that the treatment should not be allowed while keeping the treated tunnels and greenhouses open. Greenhouses should be kept closed until 18d post-treatment.

B.6.16 References relied on**Toxicology and metabolism of the active substance (Annex IIA 5)**

Annex point / reference number	Author(s)	Date	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
IIA 5.1.1 /01	Hawkins DR, Elsom LF, Girkin R	1987-11-12	The biokinetics and metabolism of 14C-metam sodium in the rat [REDACTED] HRC/BSF 455/6/7/8875 GLP Not published	Y	USA TF
IIA 5.1.3 /01	Stewart FP	1991-10-07	Pilot dermal absorption study with 14C-Metam Sodium in female rats [REDACTED] CTL/C/2463 GLP Not published	Y	USA TF
IIA 5.1.3 /02	Stewart FP	1992-11-30	Metam-Sodium: in vivo percutaneous absorption study in the rat [REDACTED] CTL/C/2843 GLP Not published	Y	USA TF
IIA 5.1.3 /03	Clowes HM	1993-10-15	Metam sodium: in vitro absorption through rat and human skin [REDACTED] CTL/P/4118 GLP Not published	Y	USA TF
IIA 5.2.1 /01	Baldrick P, Healing G	1991-01-07	Acute Oral Toxicity to rats of metam sodium [REDACTED] HRC 901046D/UCB 366/AC GLP Not published	N	Taminco
IIA 5.2.1 /02	Sulaiman SM	2002-10-11	Acute oral toxicity study (acute toxic class method) with metam potassium aqueous solution in wistar rats [REDACTED] 3377/02 GLP Not published	Y	Taminco
IIA 5.2.1 /03	Kieczka H, Kirsch P	1986-10-15	Report on the study of acute oral toxicity of the rat based on OECD and EPA (FIFRA) of MITC [REDACTED] 86/281 Not GLP Not published	Y	BASF, Taminco
IIA 5.2.2 /01	Baldrick P, Healing G	1991-01-07	Acute dermal toxicity to rats of metam sodium [REDACTED] HRC 90955D/UCB 367/AC GLP Not published	N	Taminco
IIA 5.2.2 /02	Sulaiman SM	2002-10-11	Acute dermal toxicity study (Acute toxic Class Method) with metam potassium aqueous solution in wistar rats [REDACTED] 3378/02 GLP Not published	Y	Taminco

Annex point / reference number	Author(s)	Date	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
IIA 5.2.2 /03	Kirsch P, Hildebrand B	1987-01-15	Report on the study of the acute dermal toxicity of the rat based on OECD and EPA (FIFRA) of MITC [REDACTED] 87/014 Not GLP Not published	Y	BASF, Taminco
IIA 5.2.3 /01	Jackson GC, Hardy CJ	1992-01-08	Acute inhalation toxicity in rats – 4 hours exposure [REDACTED] HRC UCB 371/91206 GLP Not published	N	USA TF
IIA 5.2.3 /02	Gelbke H-P, Klimisch H-J	1986-11-06	Acute inhalation toxicity LC50 4 hours (rat) liquid aerosol study of metam sodium [REDACTED] 86/346 GLP Not published	N	USA TF
IIA 5.2.3 /03	Venugopala Rao K	2002-12-31	Acute inhalation toxicity study with metam potassium aqueous solution in wistar rats [REDACTED] 3379/02 GLP Not published	Y	Taminco
IIA 5.2.3 /04	Jackson GC et al	1981-04-09	Methyl isothiocyanate: Acute inhalation toxicity in rats, 4 hour exposure [REDACTED] BSF 378/801109, 81/082 GLP Not published	Y	KST, Taminco
IIA 5.2.4 /01	Liggett MP, McRae LA	1991-01-07	Skin irritation to rabbits with metam sodium [REDACTED] HRC 90997D/UCB 368/SE GLP Not published	N	Taminco
IIA 5.2.4 /02	Kirsch P, Kieczka H	1986-10-27	Report on the study of the acute dermal irritation/corrosivity to the intact dorsal skin of the white rabbit based on OECD and EPA (FIFRA) of MITC [REDACTED] 86/319 Not GLP Not published	N	KST, Taminco
IIA 5.2.4 /03	Kirsch Grundler	1982-03-01	Report on the study of the acute irritation/corrosion to the intact dorsal skin of White Rabbits [REDACTED] 88/0476 Not GLP not published	N	USA TF
IIA 5.2.4 /01	Liggett MP, McRae LA	1991-01-07	Eye irritation to rabbits with metam sodium [REDACTED] HRC 90998D/UCB 369/SE: GLP Not published	N	Taminco
IIA 5.2.5 /02	Prakash PJ	2002-10-11	Acute eye irritation/corrosion study with metam potassium aqueous solution in new zealand white rabbits	Y	Taminco

Annex point / reference number	Author(s)	Date	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
			██████████ 3381/02 GLP Not published		
IIA 5.2.6 /01	Parcell BI, Denton S M	1991-01-07	Delayed contact hypersensitivity in the guinea-pig with metam sodium ██████████ HRC 901002D/UCB 370/SS GLP Not published		Taminco
IIA 5.2.6 /02	Merkel DJ	2002-12-02	Metam potassium. Dermal sensitization study in guinea pigs (Magnusson-Kligman method) ██████████ 12086 GLP Not published	Y	Taminco
IIA 5.2.6 /03	Kieczka H, Kirsch P	1986-12-02	Report on the maximization test for the sensitizing potential of MITC in guinea pig ██████████ 86/374 Not GLP Not published	Y	KST, Taminco
IIA 5.3.1 /01	Whiles AJ	1990-12-11	Metam Sodium: pilot drinking water study in mice ██████████ CTL/L/3466 Not GLP Not published	Y	USA TF
IIA 5.3.1 /02	Cave DA	1991-01-18	Metam sodium: 21 day drinking water study in rats ██████████ CTL/T/2716 Not GLP Not published	Y	USA TF
IIA 5.3.2 /01	Whiles AJ	1991-09-26	Metam Sodium: 90 day drinking water study in mice with a 28 day interim kill ██████████ CTL/P/3185 GLP Not published	Y	USA TF
IIA 5.3.2 /02	Allen SL	1991-09-26	Metam Sodium: 90 day drinking water study in rats ██████████ CTL/P/3213 GLP Not published	Y	USA TF
IIA 5.3.2 /03	Brammer A	1992-11-11	Metam Sodium: 90-day oral dosing study in dogs ██████████ CTL/P/3679 GLP Not published	Y	USA TF
IIA 5.3.2 /04	Brammer A	1993-05-24	Metam sodium: assessment of recovery in dogs ██████████ CTL/L/5204 Not GLP Not published	Y	USA TF
IIA 5.3.2 /05	Harling RJ, Massey JE, Buist DP, Edgarm HH, Street AE, Brown G,	1986-03-25	Methyl Isothiocyanate: Oral toxicity study in Beagle Dogs (Final Report – Repeated administration for 13 weeks) ██████████ Tox/86/203-218 GLP Not published	Y	Taminco (access)

Annex point / reference number	Author(s)	Date	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
	Majeed SK, Gopinath C				
IIA 5.3.2 /06	Brammer A	1994-05-23	Metam sodium: 1 year oral toxicity study in dogs [REDACTED] CTL/P/4196 GLP Not published	Y	USA TF
IIA 5.3.3 /01	Knapp HF	1983-08-31	Subchronic inhalation study with vapam technical in rats [REDACTED] T-11006 Not GLP Not published	Y	USA TF
IIA 5.3.3 /02	Duffell SJ	1992-10-29	Metam-sodium: a CTL re-examination of liver sections from a sub-chronic inhalation study with vapam technical in rats ICI CTL, CTL/T/2815 GLP Not published	Y	USA TF
IIA 5.3.3 /03	Leuschner F	1979-02-12	3-weeks-toxicity of Metam Fluid (methyl-dithio carbamic sodium) lot BAS 00500N – called for short ‘Metam Fluid’ – during local administration in rabbits [REDACTED] 79/0140 Not GLP Not published	Y	USA TF
IIA 5.3.3 /04	Klimisch H-J, Deckhardt K, Hasenohrl K, Hildebrand B	1987-07-03	Study on the Subchronic Inhalation Toxicity of Methyl Isothiocyanate in Wistar Rats (4-Week Study) [REDACTED] 87/0244 (translation of report 87/039) GLP Not published	Y	USA TF
IIA 5.4.1 /01	Mackay JM	1996-10-14	Review of metam sodium genetic toxicology CTL, CTL/P/5287 GLP Not published	Y	USA TF
IIA 5.4.1 /03	Engelhardt G	1987-06-05	Report on the Study of Metam Sodium in the AMES test BASF, 87/0208 GLP Not published	N	USA TF
IIA 5.4.1 /04	Gelbke H-P, Engelhardt G	1986-01-28	Report on the study of Methylisothiocyanate in the Ames test BASF AG, 83/010 Not GLP Not published	Y	KST, Taminco
IIA 5.4.1 /05	Jagannath DR	1989-03-13	Report on the mutagenicity test on MITC in the recombination assays with bacillus subtilis strains H17 (rec+) and M45 (rec-) Hazleton Laboratories, 10538-0-404, BASF 89/0098 GLP Not published	N	KST, Taminco

Annex point / reference number	Author(s)	Date	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
IIA 5.4.1 /06	Engelhardt G	1987-08-03	Report on the study for gene mutations in vitro of Metam sodium in Chinese Hamster Ovary Cells (HGPRT locus) with and without metabolic activation BASF, 87/0280 GLP Not published	Y	USA TF
IIA 5.4.1 /07	Gelbke HP, Engelhardt G	1987-03-09	Report on the in vitro cytogenetic investigations in human lymphocytes with metam sodium BASF, 87/0116 GLP Not published	N	Taminco
IIA 5.4.1 /08	Mackay JM	1996-06-17	Metam sodium: in vitro cytogenetic assay in human lymphocytes CTL, CTL/E/0103 GLP Not published	Y	USA TF
IIA 5.4.1 /09	Engelhardt G, Gelbke H-P	1987-05-26	Report on the in vitro cytogenetic investigations in human lymphocytes with Methylisothiocyanate BASF AG, 87/0184 GLP Not published	Y	BASF, Taminco
IIA 5.4.1 /10	Nihon Schering K.K., Shionogi & Co	1990-02-20	Summary of Toxicity data on Methyl Isothiocyanate (MITC) Agrochemical div., Nihon Schering K.K. and Shionogi & Co., Ltd. Japan, J. Pesticide Sci. 15, 297-304 Not GLP Published	N	publication
IIA 5.4.1 /11	Cifone MA	1987-07-01	Report on the Mutagenicity test on metam sodium in the rat primary hepatocyte unscheduled DNA Synthesis assay Hazleton, 9736-0-447, BASF 87/0240 GLP Not published	Y	USA TF
IIA 5.4.1 /12	Hoorn AJW	1987-03-27	Report on the mutagenicity test on metam-sodium in the REC-Assay with Bacillus Subtilis Hazleton, BASF 87/0163 (HLA) GLP Not published	N	USA TF
IIA 5.4.2 /01	Barber G, Mackay JM	1996-04-18	Metam sodium: an evaluation in the mouse micronucleus test (in vivo) ██████ CTL/E/0104 GLP Not published	Y	USA TF
IIA 5.4.2 /02	Allen JA, Proudlock RJ, Pugh LC	1985-12-13	Technical methylisothiocyanate (MITC) : Mouse micronucleus test ████████████████████ TOX85051, TOX/85/203-2 GLP Not published	Y	Taminco (access)

Annex point / reference number	Author(s)	Date	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
IIA 5.4.2 /03	Gelbke HP, Engelhardt G	1987-06-30	Cytogenetic study in vivo of Metam-Sodium in Chinese hamsters bone marrow chromosome analysis, single oral administration [REDACTED] 87/0238 GLP Not published	Y	USA TF
IIA 5.5.1/01	Allen SL	1991-03-28	Metam Sodium: Pilot drinking study in rats [REDACTED] CTL/L/3844 Not GLP Not published	Y	USA TF
IIA 5.5.1 /02	Rattray NJ	1994-05-23	Metam Sodium: Two year drinking study in rats [REDACTED] CTL/P/4139 GLP Not published	Y	USA TF
IIA 5.5.1 /03	Rattray NJ	1994-07-11	First amendment to metam sodium: 2 year drinking study in rats [REDACTED] CTL/P/4139 GLP Not published	Y	USA TF
IIA 5.5.1 /04	Brown D	1984-02	T27/2 Methyl isothiocyanate: a chronic oral (drinking water) toxicity and carcinogenicity study in the rat (part 1 and 2) [REDACTED], 2611-14/1R Not GLP Not published	Y	Taminco (access)
IIA 5.5.1 /05	Brown D	1988-06	A chronic oral (drinking water) toxicity and carcinogenicity study in the rat – addendum [REDACTED] 2611-14/1R GLP Not published	Y	Taminco (access)
IIA 5.5.1 /06	Iwan J, Kaiser R, Hollihn U	1988-10-12	T27/2 Rat Chronic/Oncogenicity. Answers to questions posed by EPA reviews Schering AG, T27/2 Not GLP Not published	Y	Taminco (access)
IIA 5.5.2 /01	Horner SA	1994-04-20	Metam sodium: Two year drinking study in mice [REDACTED] CTL/P/4095 GLP Not published	Y	USA TF
IIA 5.5.2 /02	Pistel F	1994-10-02	Assessment of the carcinogenic potential of Metam sodium SCC, 152-001 Not GLP Not published	Y	Taminco
IIA 5.5.2 /03	Satoh R et al	1980-12	Methyl isothiocyanate (MITC): 2 year oral toxicity and oncogenicity study with methyl isothiocyanate in albino mice (106 week final report) [REDACTED] T52 Not GLP Not published	Y	Taminco (access)

Annex point / reference number	Author(s)	Date	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
IIA 5.5.2 /04	Shigeshi Toyoshima	1982	Methyl isothiocyanate: Two-year oral toxicity study in albino mice (106-week final report), Volume III – stability test of methyl isothiocyanate (MITC) in animal drinking water (trials 1 and 2) ██████████ T52 Vol III Not GLP Not published	Y	Taminco (access)
IIA 5.5.2 /05	Shigeshi Toyoshima	1982	Methyl isothiocyanate: Two-year oral toxicity study in albino mice (106-week final report), Volume IV – stability test of methyl isothiocyanate (MITC) in animal drinking water (trials 3 and 4) ██████████ T52 Vol IV Not GLP Not published	Y	Taminco (access)
IIA 5.5.2 /06	Kashima M, Sato H	1985-04	Methyl isothiocyanate: two-year oral toxicity in albino mice (106-week final report) Addendum to final report ██████████ T52 addend Not GLP Not published	Y	Taminco (access)
IIA 5.5.2 /07	Hiraide Y	1988-08-29	Methyl isothiocyanate: two-year oral toxicity study in albino mice T52 amend 1, T52 addendum ██████████ T52 amend, T52 addendum Not GLP Not published	Y	Taminco (access)
IIA 5.6.1 /01	Milburn GM	1993-12-23	Metam sodium: multigeneration study in the rat ██████████ CTL/P/3788 GLP Not published	Y	USA TF
IIA 5.6.1 /02	Barker L	1988-12-29	Technical methyl isothiocyanate (MITC): 2 generation oral (drinking water) reproduction study in the rat T98 (part 1 and 2) ██████████ T98 TOX 87/203-21, 5289-194/8 GLP Not published	Y	Taminco (access)
IIA 5.6.1 /03	Jackson CM	1988-04-12	Technical methyl isothiocyanate (MITC) 2 generation oral (drinking water) reproduction study in the rat – addendum to the report ██████████ T 98 TOX 87/203-21 Not GLP Not published	Y	Taminco (access)
IIA 5.6.1 /04	Barker L	1986-02-13	T98A Technical methyl isothiocyanate (MITC): 2 generation oral (drinking water) reproduction study in the rat -Chemistry report ██████████ Report No 5289-194/8 Not GLP Not published	Y	Taminco (access)

Annex point / reference number	Author(s)	Date	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
IIA 5.6.2 /01	Hellwig J, Hildebrand B	1987-03-25	Report on the study of the prenatal toxicity of metam-sodium in rats after oral administration (gavage) + supplement ██████████ 87/0128 GLP Not published	Y	USA TF
IIA 5.6.2 /02	Rose	1991-12	Supplemental information on Historical Control Skeletal Variations in the Metam Sodium Rat Teratology Study Addendum to MRID 41577101 ██████████ 87/0128 Not GLP Not published	Y	USA TF
IIA 5.6.2 /03	Tinston DJ	1993-05-06	Metam sodium : dose range finding study in pregnant rat ██████████ CTL/T/2823 GLP Not published	Y	USA TF
IIA 5.6.2 /04	Tinston DJ	1993-10-05	Metam sodium: developmental toxicity study in the rat ██████████ CTL/P/4052 GLP Not published	Y	USA TF
IIA 5.6.2 /05	Hellwig J, Hildebrand B	1987-09-02	Report on the study of the prenatal toxicity of MITC in rats after oral administration (gavage) ██████████ BASF 87/0326 GLP Not published	Y	BASF, Taminco
IIA 5.6.2 /06	Hodge MCE	1993-03-30	Metam sodium : dose range finding study in the rabbit ██████████ CTL/L/5197 Not GLP Not published	Y	USA TF
IIA 5.6.2 /07	Hodge MCE	1993-05-24	Metam-sodium: Dose Range Finding Study In The Pregnant Rabbit ██████████ CTL/T/2828 GLP Not published	Y	USA TF
IIA 5.6.2 /08	Hodge MCE	1993-09-06	Metam sodium: developmental toxicity study in the rabbit ██████████ CTL/P/4035 GLP Not published	Y	USA TF
IIA 5.6.2 /09	Hellwig J	1987-07-15	Report on the study of the prenatal toxicity of metam-sodium in rabbits after oral administration ██████████ 87/0255 GLP Not published	Y	USA TF
IIA 5.6.2 /10	Becker H, Sachsse K, et al.	1986-09-05	Report on the study of the embryotoxicity (including teratogenicity) in the rabbit with MITC	Y	BASF, Taminco

Annex point / reference number	Author(s)	Date	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
			Ittingen, 056687, BASF 86/395 GLP Not published		
IIA 5.8.1 /01	Nihon Schering K.K. and Shionogi & Co.	1990-02-20	Summary of Toxicity data on Methyl Isothiocyanate (MITC) Agrochemical div., Nihon Schering K.K. and Shionogi & Co., Ltd. Japan, J. Pesticide Sci. 15, 297-304 Not GLP Published		publication
IIA 5.8.2/01	Lamb IC	1993-01-06	An Acute Neurotoxicity Study of Metam Sodium In Rats WIL-188009 GLP Not published	Y	USA TF
IIA 5.8.2/02	Lamb IC	1993-09-27	The Effect of Metam-Sodium On Cholinesterase Following Acute Oral Exposure In The Rat WIL-188010 GLP Not published	Y	USA TF
IIA 5.8.2/03	Allen SL	1994-04-29	Metam Sodium: Subchronic Neurotoxicity Study in Rats CTL/P/4334 GLP Not published	Y	USA TF
IIA 5.8.2/04	Allen SL	1994-04-20	Metam Sodium: Subchronic Neurotoxicity Study in Rats - Individual Animal Data Supplement CTL/P/4334 GLP Not published	Y	USA TF
IIA 5.8.2/05	Allen SL	1995-07-25	First Supplement to Metam Sodium: Subchronic Neurotoxicity Study in Rats CTL/P/4334 GLP Not published	Y	USA TF
IIA 5.8.2/06	Allen SL	1995-10-17	Second Supplement to Metam Sodium: Subchronic Neurotoxicity Study in Rats CTL/P/4334 Not GLP Not published	Y	USA TF
IIA 5.9.2 /01	Cone et al.	1994	Persistent Respiratory Health Effects After a Metam Sodium Pesticide Spill University of California, Chest 1994, 106(2):500-508 Not GLP Published	N	publication
IIA 5.9.3 /01	Kreutzer RA, Hewitt DJ and Draper WM	1993-03-16	An epidemiological Assessment of the Cantara Metam Sodium Spill California Dep. of Health, Emeryville and California Department of Health Services, Berkely, Environmental epidemiology 15, p209-	N	publication

Annex point / reference number	Author(s)	Date	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
			230 Not GLP Published		

Toxicology and metabolism of the formulations (Annex III A 7)

Annex point / reference number	Author(s)	Date	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
IIIA 7.2.1/ 01	Mulder A, Roosjen Js, Dijksterhuis A	1994	Blootstelling van de toepasser aan methylisothiocyanaat tijdens de grondontsmetting met metam-natrium in het fabrieksaardappelgebied (<i>Exposure of the user to methylisothiocyanate during soil decontamination with Metam-sodium on industrial potato land</i>) Hilbrands laboratorium voor bodemziekten, 9410 Not published Not GLP	Y	Taminco, Cerexagri
IIIA 7.2.1 /02	de Rooij M, Dijksterhuis A, van Aartrijk J	1993-03	Blootstelling van de toepasser aan methylisothiocyanaat tijdens de grondontsmetting van bloembollenpercelen met metam-natrium (<i>Exposure of the user to methylisothiocyanate during soil decontamination of bulb parcels with Metam-sodium</i>) Stichting laboratorium voor bloembollen-onderzoek Lisse Not published Not GLP	Y	Taminco
IIA 7.2.2 IIIA 7.2.1.2 IIIA 9.3	Links, IHM; Veldhof, RJG; Lurvink, MWM; Schouten, A; van Hemmen, JJ	2006-03-16	Operator exposure to methyl isothiocyanate (MITC) and methyl isocyanate (MIC), and air monitoring of MITC, MIC, carbon disulfide and methylamine following use of Metam-sodium TNO Zeist, V6654/01 GLP Not published	Y	Taminco
IIIA 7.2.1 /03	Jarvis T	2004-11-18	Predicted emission rates of MITC into air in the EU using the FOCUS Groundwater Scenarios with the PRZM model Exponent International Ltd, TA18804-3 Not published Not GLP	Y	Taminco

Annex point / reference number	Author(s)	Date	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
IIIA 7.2.2 /01	Saeed IAM, Rouse DI, Harkin JM	2000	Methyl isothiocyanate volatilization from fields treated with metam-sodium Pest Manag Sci, 56:813-817 Not published GLP	N	public
IIIA 7.2.2 /02	Van den Berg F	1993	Measured and computed concentrations of methyl isothiocyanate in the air around fumigated fields DLO Winand Staring Centre, Atmospheric Environment Vol 27A: 63-71 Not published GLP	N	public
IIIA 7.2.3 /01	Schepel EG, Dijksterhuis A	2003	Operator and bystander inhalatory exposure to MITC during and after breaking of the seal after soil fumigation with Metam-sodium (including tunnel measurements) HLB, 193, project 2266 Published Not GLP	Y	Taminco
IIIA 7.2.3 /02	den Tonkelaar WAM, Heidema LF	1988-11-21	Emissie en verspreiding van methylisothiocyanaat bij grondontsmetting van kassen (Emission and spreading of methyl isothiocyanate with soil decontamination in glasshouses TNO, R 88/194A Not published Not GLP	N	Taminco, KST, Cerexagri, Bayer CS
IIIA 7.2.3 /03	Thouvenin, I	2008	Determination of operator and worker inhalation exposure and air monitoring of methyl isothiocyanate (MITC) following drip irrigation with Nemasol® 510	Y	Taminco

Published data not provided by the company and used in the DAR by the RMS:

Annex point	Authors	Title	Published
IIA 5.1.1 /01	Lam, WW, Kim JH, Sparks, SE, Quistad, G and Casida JE, 1993	Metabolism in rats and mice of the soil fumigants of metam, methyl isothiocyanate and Dazomet	J.Agric.Food Chem., 41, 1497-1502
IIA 5.2.1	Thompson, RW, Valentine, HL and Valentine WM., 2002	In vivo and in vitro hepatotoxicity and GSH interactions of N-methyldithiocarbamate in the rat.	Toxicol. Sci., 70, 269-280.
IIA 5.2.1	Stephen B. Pruett ¹ , Qiang Zheng, Carlton Schwab and Ruping Fan, 2005	Sodium Methyldithiocarbamate Inhibits MAP Kinase Activation through Toll-like Receptor 4, Alters Cytokine Production by Mouse Peritoneal Macrophages, and Suppresses Innate Immunity	Toxicological Sciences 87(1) :75-85

Annex point	Authors	Title	Published
IIA 5.3	Brittebo, E., 1997	Metabolism-dependent activation and toxicity of chemicals in nasal glands.	Mutation Research 380, 61-75
IIA 5.3	California Environmental Protection Agency, 2004	Metam sodium risk characterization document. Medical toxicology branch Department of Pesticide Regulation	-
IIA 5.3	Harkema, JR, 1990	Comparative pathology of the nasal mucosa in laboratory animals exposed to inhaled irritants	Environ. Health Perspect. 85, 1990, 231-238
IIA 5.3	Gaskell, BA, 1990	Nonneoplastic changes in the olfactory epithelium-experimental studies	Environ. Health Perspect. 85, 1990, 275
IIA 5.3	Morgan, DL., price, H., O'Connor, R., Seely, JC., Ward, SM, Wilson, RE., and Cunningham, M, 2000	Upper respiratory tract toxicity of inhaled methylvinyl ketone in F344 rats and B6C3F1 mice	Toxicol Sci, 58, 182-194.
IIA 5.4	Fekadu Kassie, Brenda Laky, Eva Nobis, Michael Kundi and Siegfried Knasmüller, 2001	Genotoxic effects of methyl isothiocyanate	Mutation Research/Genetic Toxicology and Environmental Mutagenesis Volume 490, (1) , 25 , 1-9
IIA 5.5.2	Joan E. Denton, 2003	Comments and recommendations on the Draft Metam sodium risk characterization document prepared by the department of pesticide regulation California Environmental Protection Agency Memorandum to: Gary T. Patterson, Ph.D., Chief Medical Toxicology Branch Department of Pesticide Regulation 830 K Street Sacramento, California 95814-3510 FROM: Anna M. Fan, Ph.D., Chief Pesticide and Environmental Toxicology Section	Memorandum
IIA 5.5.2	Cohen, S.M., 2002	Comparative pathology of proliferative lesions of the urinary bladder	Toxicologic Pathology gy, 30, 663-671.
IIA 5.5.8	Keil, D., Padgett, E., Barnes, D. and Pruett, 1996	Role of decomposition products in sodium methyldithiocarbamate induced immunotoxicity	J.toxicol. Environ Health, 5 ; 47 (5) :479-492
IIA 5.5.8	Pruett et al., 1992	Immunotoxicological characteristics of sodium methyldithiocarbamate	Fundam. Appl. Toxicol, 18(1)40-17
IIA 5.6.2	Goldman et al., 1994	Blockade of ovulation in the rat by the fungicide metam sodium: relationship between effects on the luteinizing hormone surge and alterations in hypothalamic catecholamines	Neurotoxicology and Teratol., 16 (3), 257-268

Annex point	Authors	Title	Published
IIA 5.8.1	John R. Froines, 2002	The Department of Pesticide Regulation's document: Evaluation of methyl isothiocyanate (MITC) as a toxic air contaminant. Regulation's draft report titled "Evaluation of Methyl Isothiocyanate (MITC) as a Toxic Air Contaminant").	Scientific Review Panel, California, based on the Panel's review of the Department of Pesticide
IIA 5.8.1	1997	State of California, California Environmental Protection Agency AIR RESOURCES BOARD, Ambient Air Monitoring for MIC and MITC after a Soil Injection Application of Metam Sodium in Kern County during August 1995.	Engineering and Laboratory Branch Monitoring and Laboratory Division Test Report No. C94-046A Report
IIA 5.8.1	G. A. Boorman, R. Brown, B. N. Gupta, L. C. Uraih and J. R. Bucher. 1987	Pathologic changes following acute methyl isocyanate inhalation and recovery in B6C3F1 mice	Toxicology and Applied Pharmacology , 87, Issue 3 , 446-456
IIA 5.8.1	Mitsumori, K, Boorman, G.A., Gupta, B.N., and Bucher, J.R. 1987	Four-day repeated inhalation and recovery study of methyl isocyanate in F344 rats and B6C3F1 mice.	Fund. Appl Toxicol 9:480-495
IIA 5.8.1	Sethi N, Dayal R, Singh RK, 1989	Acute and subacute toxicity study of inhaled methyl isocyanate in Charles Foster rats.	Ecotoxicol Environ Saf. 1989 , 18(1):68-74
IIA 5.8.1	Schwetz, et al, 1987	Methylisocyanate: reproductive and developmental toxicology studies in Swiss mice.	Environ Health perspect., 72, 149-152
IIA 5.8.1	Shelby, MD, Allen, JW, Caspary, W, Haworth, S., Ivett, J., Kligerman, A., Luke, CA, Mason, J., Myhr, B., Tice, RR., Valencia, R and Zeiger, E. 1987	Results of in vitro and in vivo genetic toxicity tests on methyl isocyanate	Environ Health perspect., 72, 183-187
IIA 5.8.1	David W. Herr, Jaimie E. Graf, Virginia C. Moser, Kevin M. Crofton, Peter B. Little, Daniel L. Morgan and Robert C. Sills.	Inhalational Exposure to Carbonyl Sulfide Produces Altered Brainstem Auditory and Somatosensory-Evoked Potentials in Fischer 344N Rats	Toxicological Sciences 2007 95(1):118-135
IIA 5.9.2	O' Malley, Barry, Verder-Carlos and Rubin, 2004	Modeling of MITC air concentrations associated with community illnesses following metam sodium sprinkler application	Am J Ind Med.;46(1):1-15
IIA 5.9.2	Bowler et al. 1994	Aftermath of a Chemical Spill.	Psychological and Physiological Sequelae, NeuroToxicology, 15(3): 723 -730)

Annex point	Authors	Title	Published
IIA 5.9.3	O'Malley M, Barry T, Ibarra M, Verder-Carlos M, Mehler L., 2005	Illnesses related to shank application of metam-sodium, Arvin, California, 2002.	J Agromedicine. 10(4):27-42
IIA 5.9.4	Koo D, Goldman L, Baron R. 1995	Irritant dermatitis among workers cleaning up a pesticide spill: California 1991.	Am J Ind Med. 27(4):545-53.
IIA 5.9.4	Cone JE, Wugofski L, Balmes JR, Das R, Bowler R, Alexeeff G, Shusterman D, 1994	Persistent respiratory health effects after a metam sodium pesticide spill.	Chest, 106(2); 500-5008

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Appendix 1: Discussion of the “comparative table of the batches used in the toxicological studies and the proposed specification or justification to waive it”, as proposed by the notifier

The notifier submitted following argumentation in a position paper, in order to justify the lack of information about the impurities in the batches tested in the toxicity studies (reference made to impurities were adapted for confidentiality reasons):

“

- All acute toxicity studies with **metam-sodium** presented in the above table (except acute inhalation) have been performed with a sample of Metam-sodium 510 g/L produced by **UCB**, which is currently **Taminco**, the main applicant. Although there are no specific data available, there are no reasons to assume the impurity pattern in the test products is significantly different from the product currently produced.
- All acute toxicity studies with **metam-potassium** have been performed with a sample of metam-potassium 690 g/L by **UCB/Taminco**. The TNO 5-batch analysis report dates from the same period (2002) therefore there are no reasons to assume the impurity pattern of the test product used for the acute toxicity studies would be significantly different from the specifications of the product currently manufactured.
- A high number of studies has been performed with test product from **BASF**, a former producer of **metam-sodium** and a former member of the US Metam-sodium Task Force. Data was shared in the context of this task force.
 - o A 21 day dermal toxicity study with rabbit performed in 1979. A batch number is not known but the product is called 005 00N which is a BASF code for metam-sodium 510 g/L aqueous solution. The purity given is 42.2 %, which is consistent with the specification proposed by the applicant. A technical product specification of 1983 is available showing that the higher concentrated product (600 g/L) contained max. 0.21% w/w of impurity 1. BASF used to produce the 510 g/L formulation by dilution of the 600 g/L technical product.
 - o The acute inhalation study, a series of genotoxicity studies and developmental toxicity studies in rabbits from 1986-1987 were carried out with a test product named “Metam Fluid 510 g/L” or 005 00N, with batch number ZH 130 858, a declared purity of 42.2 % or 517 g/L. From two of the 1987 genotoxicity studies it is known that this specific batch of BASF test product contains 56.8 % water and 1.0 % of impurity 1. The active ingredient content is in line with the specification proposed by the applicant whereas the amount of impurity 1 was higher (1.0 % in test product vs. 0.29% specification Taminco).
 - o Subchronic toxicity studies in mice, rat and dog, the 2 year rat and mice studies, the 2 generation study in rat, developmental toxicity studies in rat and rabbit and the subchronic neurotoxicity study have been carried out in 1991-1993 with BASF product (code 005 00N, batch numbers Y06930/007 or 008), with purity 525.54 g/L or 43.15 %. The a.i. content is in line with the specifications proposed by the applicant. In 1992, samples of Metam-sodium 510 g/L from UCB and BASF source have been compared to determine if they were comparable. A letter from Dr. Genari (BASF) is attached Annex 2). Another analysis from 1993 by G. Matthijs (UCB) confirms that the specifications of UCB and BASF for impurity 1 [...] were comparable (Annex 3).
- The 90 d inhalation toxicity study was carried out with a sample from Stauffer, 1983 (afterwards Zeneca, now Amvac). The a.i. content is lower than in the technical product produced by the applicant (Taminco), nl. 32.7 % however the use of this diluted form is not expected to influence the result which is expressed as a.i.. Zeneca and Amvac are (former) members of the USA Metam-sodium Task Force.
- The CA in human lymphocytes and in vivo mouse micronucleus test (1996) were done with samples from Zeneca WRC (now Amvac). The a.i. content is lower than in the technical product produced by

the applicant (Taminco), nl. 32.3 % however the use of this diluted form is not expected to influence the result which is expressed as a.i.. Zeneca and Amvac are (former) members of the USA Metam-sodium Task Force.

- *Test product of MITC was of very high purity (>95%). In the degradation of metam, MITC is a metabolite therefore it is not relevant to compare specifications of the test product with the metabolite formed after application.*
- *None of the former analytical work or product specifications mention the impurity 2 [...], which is included in the analysis of metam itself. The 5-batch analysis generated on behalf of Taminco in 2002 was the first time where the impurity 2 was quantified. So this new specification is a result of improved analytical techniques but does not imply this impurity was not present in previous test products.*
- *In conclusion, toxicological studies have been conducted with test material from different members of the US Metam Sodium Task Force. The metam test material used for these studies is all produced starting from the same raw materials and through the same basic process. The detailed composition of the test material is however not always known to Taminco. However, US EPA has declared all registered sources equivalent and the toxicological and ecotoxicological studies are supporting or have supported the various US registrations (currently Taminco, Amvac, Buckman, TKI and in the past Zeneca/Stauffer and BASF). In addition, the manufacturing procedures have not significantly changed since more than 30 years."*

Appendix 2: Short overview of the techniques of application of the soil fumigant Metam (explanation provided by the notifier)



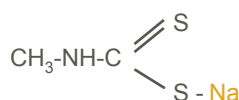
Short overview of the techniques of application of the soil fumigant METAM



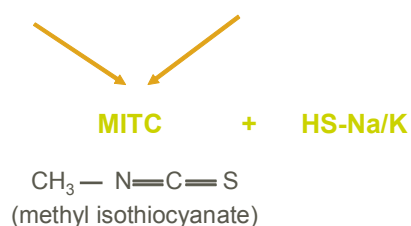
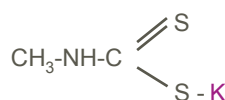
Background info

Metam, in contact with soils, rapidly degrades to methylisothiocyanate, which has disinfecting properties. It should be kept in mind that Metam/MITC is not a conventional plant protection product, because it is used to treat the bare soil before planting or sowing. It never really enters into contact with the crop.

metam sodium



metam potassium



Intended Uses and Methods of Application

Metam is applied as an aqueous solution on an idle soil, before installation of the crop by

- By drip irrigation
- By mechanical injection

The treatment is done in preparation of the soil before cultivation of

Field crops : by mechanical injection

Carrots
Corn salad
Potato
Strawberry
Grape
Tomato

Greenhouse crops: by drip irrigation

Cucumber
Eggplant/aubergine
Pepper
Tomato

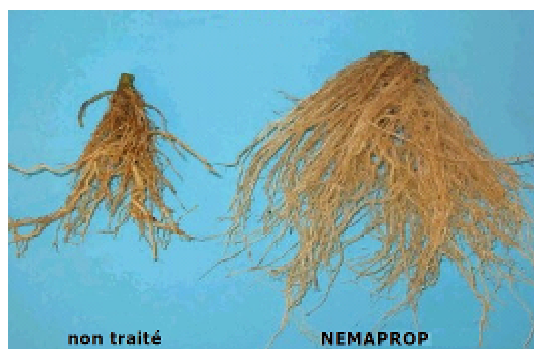
Soil fumigants are essential in intensive cropping. Metam is of high importance for the growers, as herbicide, nematicide and fungicide :

One application permits a preventive control of soil populations of:

- nematodes: *Meloidogynae*, *Ditylenchus*, *Heterodera*, *Pratylenchus*...
- soil diseases: *Fusarium*, *Verticillium*, *Pythium*, *Armillaria*...
- weeds: *Solanum*, *Cyperus*, *Orobanchae*, monocot, dicot



Herbicide



Nematicide



Fungicide

After 14-21 days, the crop is planted or sown, assuming soil temperatures higher than 12°C. In regions or climates with lower soil temperatures, longer waiting periods up to 45 days are recommended in function of the soil temperature.

After this waiting period, no metam or MITC is present in the soil.

As remaining MITC could be phytotoxic, a germination test (cress) is done before planting or sowing.



Application



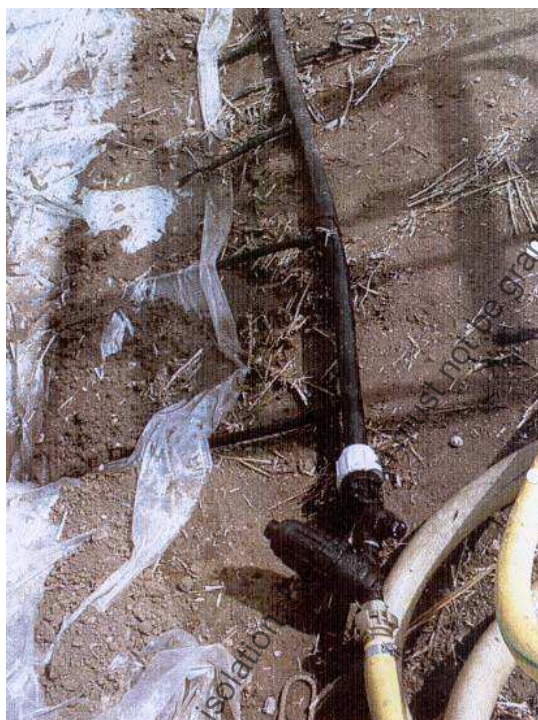
2-3 weeks



Planting

Application by drip irrigation

Use is made of the existing irrigation network in greenhouses and/or under plastic sheeting. An automatic pumping system dilutes Metam aqueous solution with the desired quantity of water. The dilution factor is based on the application rate per ha of treated soil surface and the quantity of water normally needed to irrigate the soil to a certain depth (root area).



There is virtually no contact to the product: in the beginning of the application the operator (mixer/loader) has to dip a tube in the drum of –undiluted– metam-sodium solution. Then a pump is turned on, which is automatically diluting the metam and sending it to the irrigation tubes. At the end of the application, the operator removes the tube from the drum and puts it in a bucket of clear water. This way, the entire system is rinsed with water, which is necessary because of the phytotoxic nature of metam.



Mechanized application

Specialised equipment injects the metam-sodium aqueous solution (undiluted) directly into the soil. There is a trend towards using tractors equipped with pressurised cabins. The loading of the tank is done by pumping the product directly from the drum or container. There is no mixing and virtually no contact with the product.



Dry coupling



Direct automated transfer from container to application machine.



Tractor mounted soil injector (Rumtstadt machine) with roller.



Tractor mounted soil injector/rotavator with roller (Imants)

Modern machines are already equipped with a fixed pumping system and pumping tubes (attached to the machine).

In the latest system for transferring to product, a 500 litre drum is installed automatically on the machine (no heavy weight for the worker) and fitted with a dry coupling system (no leakage).



Tractor with metam container.