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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

MAY 1 1995

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Carcinogenicity Peer Review of Metam Sodium

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and

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THROUGH: Stephanie R. Irene Ph.D.
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The Health Effects Division Carcinogenicity Peer Review Committee (CPRC) met on March 01, 1995 to discuss and evaluate the weight-of-the-evidence on metam sodium with particular reference to its carcinogenic potential. The CPRC concluded that metam sodium should be classified as a Group B2 - probable human carcinogen, based on statistically significant increases in malignant angiosarcomas in both sexes of the CD-1 mouse, supported by a similar tumor type (malignant hemangiosarcomas) in male Wistar rats. The CPRC recommended that for the purpose of risk characterization, a low dose extrapolation model be applied to the animal data for the quantification of human risk (Q_1^*), based on the total incidence of angiosarcomas in male mice, at all sites combined.

SUMMARY

Administration of metam sodium in the drinking water to CD-1 mice resulted in statistically significant increases in angiosarcomas in the liver and spleen (the most relevant target tissues), as well as at all sites combined, at a dose of 27.2 mg/kg/day (HDT) in male mice. There was also a statistically significant increasing trend for these tumors. In female mice there was a statistically significant increase in angiosarcomas in the spleen at the HDT (29.9 mg/kg/day) and at the mid-dose (8.7 mg/kg/day). The increase in angiosarcomas in the female liver was of borderline significance (p=0.055). There were statistically significant increasing trends for these tumors in both the female liver and spleen (major target tissues). Dosing in the mouse study was considered to be adequate for assessing carcinogenic potential.

Administration of metam sodium in the drinking water to Wistar Tox rats was associated with a statistically significant increase in hemangiosarcomas at the low and mid-doses (1.3 and 3.9 mg/kg/day, respectively) but not at the HDT (12.0 mg/kg/day) in male rats. There was no apparent increase in tumor incidence in female rats. Dosing in the rat study was considered to be adequate for assessing carcinogenic potential.

Metam sodium was negative in several mutagenicity assays (including the Salmonella assay, an unscheduled DNA synthesis assay, and an aberrations assay with Chinese hamsters). However, metam sodium demonstrated a dose-dependent and statistically significant increase in the number of chromosomally damaged cells ± metabolic activation in an *in vitro* cytogenetics assay with cultured human lymphocytes. Metam sodium is structurally related to ziram and thiram, and related via a common metabolic end-product (MITC) to dazomet. There is some evidence for carcinogenic potential for all these analogs (including MITC). Carbon disulfide, another metabolite of metam sodium, also has some evidence of carcinogenicity.

The classification of Group B2 was based on increases in malignant angiosarcomas, by both pair-wise and trend analysis, in both sexes of the mouse. The angiosarcomas were also a contributing factor to death in the mice. Additional evidence included the finding of similar tumors in the male rat (malignant hemangiosarcomas) which were considered to be supportive of the tumors seen in the mouse, as well as some evidence of mutagenicity and limited evidence of carcinogenicity from structurally related analogs.

A. Individuals in Attendance at the meetings:

1. Peer Review Committee: (Signatures indicate concurrence with the peer review unless otherwise stated.)

Stephanie Irene

William Burnam

Karl Baetcke

Marcia Van Gemert

Kerry Dearfield

Elizabeth Doyle

Marion Copley

Esther Rinde

Yin Tak Woo

2. Reviewers: (Non-committee members responsible for data presentation; signatures indicate technical accuracy of panel report.)

Timothy McMahon[1] 4/6/95

Yiannakis Ioannou

Lucas Brennecke[2]
(PAI/ORNL)

3. Other Attendees:

Raymond Locke (HED) and Amber Aranda (OGC)

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[1]Also a member of the PRC for this chemical; signature indicates concurrence with the peer review unless otherwise stated.

[2]Signature indicates concurrence with pathology report.

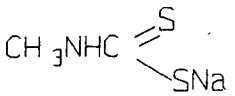
B. Material Reviewed

The material available for review consisted of DER's, one-liners, data from the literature and other data summaries prepared and/or supplied by Dr. Timothy McMahon, and tables and statistical analysis by Lori Brunsman. The material reviewed is attached to the file copy of this report.

C. Background Information

Metam Sodium (sodium-N-methyldithiocarbamate), also known as Vapam, Metham Sodium, and SMDC is a fumigant-type pesticide used as a non-selective preplant fumigant for control of weeds, nematodes, fungi, bacteria, and insects. There are approximately 35 different products containing metam sodium in concentrations ranging from 18-42% active ingredient. Use patterns for these various formulations include agricultural preplant soil fumigation, wood preservative, slimicide, tree root killer, and aquatic weed control. Approximately 10 million pounds of active ingredient were used in 1990, with 40-45% for agricultural purposes. For control of weeds, soilborne diseases, and nematodes infesting field and vegetable crops, the pesticide is applied at least 14 to 21 days prior to planting. As a slimicide, metam sodium is sprayed inside sewer mains and drain pipes; wood preservative uses involve injection of standing utility poles to control wood-destroying insects and to arrest wood rot.

As a result of the July 14, 1991 railcar accident in which thousands of gallons of metam sodium were spilled into the Sacramento River near Dunsmuir, California, the Environmental Protection Agency negotiated a settlement with the Metam Sodium Task Force. As per the settlement agreement and for reregistration purposes, the Task Force was required to conduct several toxicology and exposure studies. The toxicology data in this memorandum are based upon these submitted studies. Metam Sodium has not been previously subjected to peer review by the Health Effects Division Carcinogenicity Peer Review Committee.



Structure of Metam Sodium

D. Evaluation of Carcinogenicity Evidence

1. Two Year Carcinogenicity Study in Mice

Reference: Horner, S.A. (1994): Metam Sodium: Two Year Drinking Study in Mice. Study # PMO841. Study Conducted by Zeneca Central Toxicology Laboratory, Cheshire, UK. MRID # 432335-01

a. Experimental Design

In a two year carcinogenicity study in mice, Metam Sodium technical (43.15% active ingredient) was administered in the drinking water to groups of 55 male and 55 female C57BL/10JfCD-1/Alpk mice for 104 weeks at nominal dose levels of 0 and 0.019 mg/ml (**1.6 mg/kg/day** in males, **2.3 mg/kg/day** in females) 0.074 mg/ml (**6.5 mg/kg**/day in males, **8.7 mg/kg/day** in females) and 0.23 mg/ml (**27.7 mg/kg**/day in males, **29.9 mg/kg/day** in females).

b. Discussion of Tumor Data

Carcinogenic potential was evidenced in this study by an increase in the incidence of angiosarcoma of the liver, spleen, subcutaneous tissue, and bone marrow of the femur and spine, as well as the presence of a transitional cell papilloma in a single high dose male mouse and a transitional cell carcinoma in a single high dose female mouse. Tumorigenic evidence observed in this study is shown in the following tables (1-4) as extracted from the Qualitative Risk Assessment memorandum:

Table 1. Metam Sodium - C57BL/10JfCD-1/Alpk Mouse Study
Male Angiosarcoma Tumor Rates[+] and Exact
Trend Test and Fisher's Exact Test Results (p values)

	Dose (mg/kg/day)			
	0	1.6	6.5	27.7
Liver	1/52	8/52	5/55	10[a]/52
(%)	(2)	(15)	(9)	(19)
p =	0.023*	0.016*	0.116	0.004**
Spleen	6/53	3/53	10/55	21[b]/53
(%)	(11)	(6)	(18)	(40)
p =	0.000**	0.244[n]	0.233	0.001**
Bone Marrow	3/53	3/53	8[c]/55	15/53
(Femur) (%)	(6)	(6)	(15)	(28)
p =	0.000**	0.661	0.113	0.002**
Bone Marrow	2/53	0/53	0/55	7[d]/53
(Spine) (%)	(4)	(0)	(0)	(13)
p =	0.001**	0.248[n]	0.239[n]	0.080
Subcutaneous	1/53	1/53	2[e]/55	5/53
Tissue (%)	(2)	(2)	(4)	(9)
p =	0.020*	0.752	0.514	0.103
All Other Sites[#]	1/53	3/53	5[f]/55	9/53
(%)	(2)	(6)	(9)	(17)
p =	0.004**	0.309	0.112	0.008**
All Sites	7[g]/52	12[g]/52	12[g]/55	27[g]/52
Combined (%)	(13)	(23)	(22)	(52)
p =	0.000**	0.155	0.191	0.000**

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before week 53.
[n]Negative change from control.
[a]First liver angiosarcoma observed at week 68, dose 27.7 mg/kg/day.
[b]First spleen angiosarcoma observed at week 68, dose 27.7 mg/kg/day.
[c]First bone marrow (femur) angiosarcoma observed at week 69, dose 6.5 mg/kg/day.
[d]First bone marrow (spine) angiosarcoma observed at week 88, dose 27.7 mg/kg/day.
[e]First subcutaneous tissue angiosarcoma observed at week 71, dose 6.5 mg/kg/day.
[f]First angiosarcoma at any other site observed in the sternum at week 73, dose 6.5 mg/kg/day.
[g]Four, five, nine and eighteen animals in the 0, 1.6, 6.5, and 27.7 mg/kg/day dose groups, respectively, had angiosarcomas at multiple sites.
[#]Other sites include: abdominal cavity, aorta (adjacent tissue), bone (femur), heart, limb, lung, lymph node (mesenteric), mediastinum, mesentery, spinal cord, sternum, and thoracic cavity.
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 2. Metam Sodium - C57BL/10JfCD-1/Alpk Mouse Study

Male Angioma and Angiosarcoma Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p values)

	Dose (mg/kg/day)			
	0	1.6	6.5	27.7
Angiomas[&]	2[a]/53	1/53	0/55	1/53
(%)	(4)	(2)	(0)	(2)
p =	0.378	0.500[n]	0.239[n]	0.500
Angiosarcomas[#]	7/52	12/52	12/55	27[b]/52
(%)	(13)	(23)	(22)	(52)
p =	0.000**	0.155	0.191	0.000**
Combined	8[c]/52	13/52	12/55	28/52
(%)	(15)	(25)	(22)	(54)
p =	0.000**	0.164	0.273	0.000**

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before week 53.

[n]Negative change from control.

[a]First angioma observed at week 100, dose 0 mg/kg/day.
[b]First angiosarcoma observed at week 68, dose 27.7 mg/kg/day.
[c]One animal in the 0 mg/kg/day dose group had both an angioma and an angiosarcoma.

[&]Angioma sites include: aorta (adjacent tissue), lymph node (mesenteric), and subcutaneous tissue.

[#]Angiosarcoma sites include: abdominal cavity, aorta (adjacent tissue), bone (femur), bone marrow (femur), bone marrow (spine), heart, limb, liver, lung, lymph node (mesenteric), mediastinum, mesentery, spinal cord, spleen, sternum, subcutaneous tissue, and thoracic cavity.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 3. Metam Sodium - C57BL/10JfCD-1/Alpk Mouse Study

Female Angiosarcoma Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p values)

	Dose (mg/kg/day)			
	0	2.3	8.7	29.9
Liver	0/54	0/55	1/47	4[a]/52
(%)	(0)	(0)	(2)	(8)
p =	0.005**	1.000	0.465	0.055
Spleen	0/55	2/55	4[b]/47	5/52
(%)	(0)	(4)	(9)	(10)
p =	0.028*	0.248	0.042*	0.024*
All Other Sites[#]	4/55	2/55	6[c]/47	7/52
(%)	(7)	(4)	(13)	(13)
p =	0.070	0.339[n]	0.275	0.232
All Sites Combined	4/54	2[d]/55	6[e]/47	10[e]/52
(%)	(7)	(4)	(13)	(19)
p =	0.008**	0.331[n]	0.286	0.065

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before week 48.
[n]Negative change from control.

[a]First liver angiosarcoma observed at week 61, dose 29.9 mg/kg/day.
[b]First spleen angiosarcoma observed at week 48, dose 8.7 mg/kg/day.
[c]First angiosarcoma at any other site observed in the uterus at week 48, dose 8.7 mg/kg/day.
[d]Two animals in the 2.3 mg/kg/day dose group had angiosarcomas at multiple sites.
[e]Six animals in each of the 8.7 and 29.9 mg/kg/day dose groups had angiosarcomas at multiple sites.

[#]Other sites include: bone marrow (femur), bone marrow (spine), ear/Zymbal's gland, ileum, limb, mediastinum, ovary, salivary gland, spinal cord, sternum, subcutaneous tissue, and uterus.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 4. Metam Sodium - C57BL/10JfCD-1/Alpk Mouse Study

Female Angioma and Angiosarcoma Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p values)

	Dose (mg/kg/day)			
	0	2.3	8.7	29.9
Angiomas[&]	1/55	0/55	2[a]/47	2/52
(%)	(2)	(0)	(4)	(4)
p =	0.156	0.500	0.441	0.479
Angiosarcomas[#]	4/54	2/55	6[b]/47	10/52
(%)	(7)	(4)	(13)	(19)
p =	0.008**	0.331	0.286	0.065
Combined	5/54	2/55	8/47	11[c]/52
(%)	(9)	(4)	(17)	(21)
p =	0.009**	0.211[n]	0.194	0.075

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before week 48.

[n]Negative change from control.

[a]First angioma observed at week 87, dose 8.7 mg/kg/day.
[b]First angiosarcoma observed at week 48, dose 8.7 mg/kg/day.
[c]One animal in the 29.9 mg/kg/day dose group had both an angioma and an angiosarcoma.

[&]Angioma sites include: mammary gland, subcutaneous tissue, and uterus.

[#]Angiosarcoma sites include: bone marrow (femur), bone marrow (spine), ear/Zymbal's gland, ileum, limb, liver, mediastinum, ovary, salivary gland, spinal cord, spleen, sternum, subcutaneous tissue, and uterus.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

There were significant dose-related trends in the incidence of angiosarcoma for male mice in the liver, spleen, bone marrow (femur and spine), and subcutaneous tissue. In addition, significant trends were observed for the incidence of angiosarcoma at all other sites combined (see footnote to Table), as well as for all tissue sites combined. Significant pair-wise comparisons in the incidence of angiosarcoma were observed for the liver at the 1.6 mg/kg/day dose, and for the liver, spleen, and bone marrow (femur) at the 27.7 mg/kg/day dose. For all other sites and for all tissue sites combined, significant pair-wise differences were observed at the 27.7 mg/kg/day dose (Table 1).

As shown in Table 2, the statistical significance in tumor incidence for male mice is derived from the angiosarcoma incidence and not from the angioma incidence, which was not statistically different among treated and control male mice. It is noted that a statistically significant positive trend was observed for the incidence of angiosarcoma at all sites combined and for angioma/angiosarcoma combined, as well as a statistically significant pair-wise comparison in the incidence of angiosarcoma and angioma/angiosarcoma combined for male mice at the high dose. As noted in Table 3, significant dose-related trends were noted for liver and spleen angiosarcoma, and at all tissue sites combined for female mice. Significant pair-wise comparisons were noted in the incidence of angiosarcoma for the spleen at the 8.7 and 29.9 mg/kg/day dose levels, but not for liver. In contrast to the male mice, statistical pair-wise significance for the incidence of angiosarcoma at all sites combined at the high dose was not achieved in female mice, although the incidence was increased from 7% in controls to 19% at the high dose (Table 4).

It is noted from the tumor data in this study that for the mice sacrificed while on study and those surviving to study termination, the sites of tumorigenicity were similar. It is also noted that significant tumor sites involving the bone marrow of the femur and spine were observed in male mice, but not in female mice.

The report on carcinogenicity of metam sodium in mice stated that the treatment-related increase in angiosarcoma in a variety of sites was found to be a factor contributory to death in those mice receiving 0.23 mg/ml metam sodium in drinking water. For male mice, the strongest association was observed with angiosarcoma in the spleen. In females, an association with angiosarcoma of the spleen was also observed, but was not as prominent as for males.
The report stated that the question of whether the angiosarcomas observed at multiple sites represented primary tumors with metastatic deposits or multicentric tumors was debatable. In addition, it was stated that it was technically difficult to

accurately identify the primary site of the tumor. Thus, the total number of mice with angiosarcoma regardless of site was considered appropriate as a representation of this neoplastic lesion.

Overall Incidence (%) of Mice with Angiosarcoma in Any Site

	Dose (mg/kg/day)			
	0	1.6	6.5	27.7
Males	7/52 (13)	12/52 (23)	12/55 (22)	27/52 (52)
	Dose (mg/kg/day)			
	0	2.3	8.7	29.9
Females	4/54 (7)	2/55 (4)	6/47 (13)	10/52 (19)

In males, the incidence of angiosarcoma in controls (13%) increased to 22 and 23% at the 1.6 mg/kg/day and 6.5 mg/kg/day dose levels, and to 52% at the 27.7 mg/kg/day dose level. In female mice, the incidence in controls (7%) was exceeded only by the incidence at 8.7 mg/kg/day (13%) and 29.9 mg/kg/day (19%). According to the report, there was no treatment-related change in tumor latency for this tumor type.

While the preceding discussion includes a consideration of angiosarcomas at all sites, the tumors of the liver and spleen, which are the sites at which these tumors most commonly appear, were considered the most relevant by the CPRC. In male mice, there was a statistically significant increase in angiosarcomas of the liver and spleen at the HDT. There was also a statistically significant positive trend for these tumors at both sites. In female mice there was a statistically significant increase in angiosarcomas of the spleen at the HDT; in the liver the increase was borderline (p=.055) at the HDT. There was also a statistically significant positive trend for these tumors at both sites.

A transitional cell papilloma of the urinary bladder was observed in one male mouse at the 27.7 mg/kg/day dose level, as well as a transitional cell carcinoma in one female mouse at the 27.7 mg/kg/day dose level.

c. Non-neoplastic lesions and other findings

Non-neoplastic pathology of the urinary bladder and liver was also observed in this study at the 27.7 mg/kg/day dose level.

When all mice were considered together, the liver and urinary bladder were found to be the main sites of non-neoplastic pathology. At the high dose level (27.7 mg/kg/day in males, 29.9 mg/kg/day in females), increased incidence of epithelial hyperplasia, mononuclear cell infiltration, eosinophilic/hyaline cytoplasmic inclusions, submucosal connective tissue, and submucosal hyalinization were observed in both sexes in the urinary bladder. In the liver, increased incidence of hepatocyte fat vacuolation was observed at the high dose level in both male and female mice.

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

At 27.7 mg/kg/day in males and 29.9 mg/kg/day in females, body weight gain in male mice was decreased by 14% vs control for weeks 1-13 of the study, and by 20% for weeks 1-104 of the study. Liver weight in male mice was increased by 35% over control at this dose level and was accompanied by an increase in fat vacuolation. The incidence of non-neoplastic pathology of the urinary bladder was also increased at 27.7 mg/kg/day in male mice. In female mice, non-neoplastic pathology of the urinary bladder was increased at the 29.9 mg/kg/day dose level, as was the incidence of angiosarcoma when all tissue sites were combined. There were no statistically significant effects of treatment on survival in male or female mice. Based on these effects, the high dose level of 27.7 mg/kg/day in males, 29.9 mg/kg/day in females) is considered an adequate dose for assessment of carcinogenic potential in male and female mice.

2. Two Year Carcinogenicity Study in Rats

Reference: Rattray, N.J. (1994): Metam Sodium: Two Year Drinking Study in Rats. Study # PRO838. Conducted by Zeneca Central Toxicology Laboratory, Cheshire, UK. MRID # 432758-02.

a. Experimental Design

Metam Sodium technical (43.14% active ingredient) was administered in drinking water to groups of 64 male and female Hsd/Ola: Wistar Tox rats for either 52 weeks or 104 weeks at dose levels of 0 mg/ml, 0.019 mg/ml (**1.3 mg/kg/day** in males, **2.3 mg/k/day** in females), 0.056 mg/ml (**3.9 mg/kg/day** in males, **6.2**

mg/kg/day in females), and 0.19 mg/ml (**12.0 mg/kg/day** in males, **16.2 mg/kg/day** in females).

b. Discussion of Tumor Data

Evaluation of tumor data by the California Environmental Protection Agency, Department of Pesticide Regulation indicated a possible tumorigenic effect of metam sodium at the 0.056 mg/ml dose (**3.9 mg/kg/day** in males, **6.2 mg/kg/day** in females). According to their review, the incidence of hemangiosarcoma (8/64) was increased at this dose, in relation to the control incidence (0/64) and the high dose incidence (3/64[3]). The hypothesis that this could be a positive response was based upon the positive findings in mice as well as the reasoning that the increased incidence of this tumor at 0.056 mg/ml (**3.9 mg/kg/day** in males, **6.2 mg/kg/day** in females) could be based upon the decreased body weight observed at the high dose in relation to other doses. Lower body weight has often been shown to be associated with lower tumor incidence in rats.

Evaluation of the liver and pituitary tumor data by the Science Analysis Branch (SAB), Health Effects Division (HED) showed a statistically non-significant trend ($p = 0.119$) for pituitary adenoma in male rats, as well as statistically non-significant pair-wise comparisons between treated and control male rats ($p = 0.323$, 0.234, and 0.129 for the low, mid, and high dose groups, respectively).

For liver adenoma, a statistically non-significant trend was found ($p = 0.246$) as well as a statistically non-significant pair-wise comparison of adenoma incidence among treated male rats ($p = 0.651$, 0.452, and 0.36 for the low, mid and high dose groups, respectively). For liver adenocarcinoma and adenoma/adenocarcinoma combined, similar statistically non-significant trends and comparisons were observed.

Summary of male rat blood tumor rates is shown in Table 5, as analyzed by SAB, HED. There were no statistically significant trends in tumor rates of male rats. However, there was a significant pair-wise comparison in the incidence of hemangiosarcoma in male rats at the 1.3 and 3.9 mg/kg/day dose levels in comparison to control.

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[3]Note: These denominators differ from those in Table 5, because the California analysis did not exclude animals that died before observation of the first tumor.

Table 5. Metam Sodium - Hsd/Ola: Wistar Tox Rat Study

Male Blood Tumor Rates[+] and Peto's Prevalence Test Results (p values)

	Dose (mg/kg/day)			
	0	1.3	3.9	12.0
Hemangiomas	9[a]/50	3/50	4/51	8/51
(%)	(18)	(6)	(8)	(16)
p =	0.469[n]	0.950[n]	0.899[n]	0.688[n]
Hemangiosarcomas	0/47	3/49	8[b]/50	3/51
(%)	(0)	(6)	(16)	(6)
p =	0.414	0.017*	0.004**	0.073
Combined	9/50	6/50	11[c]/51	11/51
(%)	(18)	(12)	(22)	(22)
p =	0.375	0.713[n]	0.389	0.438

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

[a]First hemangioma observed at week 56, dose 0 mg/kg/day.

[b]First hemangiosarcoma observed at week 66, dose 3.9 mg/kg/day.

[c]One animal in the 3.9 mg/kg/day dose group had both a hemangioma and a hemangiosarcoma.

[n]Negative trend or negative change from control.

Note: Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

The sites of hemangioma in the present rat study included the cervical lymph node, mesenteric lymph node, thymic lymph node, and subcutaneous tissue. Sites for hemangiosarcoma included the mesenteric lymph node, subcutaneous tissue, tail, liver, lung, and uterus. However, the preponderance of tumors were observed only in male rats.

The question of whether these tumors were observed in separate rats was addressed in the review of the rat study by the California Department of Environmental Protection (Earl Meierhenry, personal communication). This review showed that of the benign hemangiomas found, one rat in the low dose group was found to have this tumor type at 2 sites (mesenteric and thymic lymph nodes). Of the malignant hemangiosarcomas found, 2 rats in the low dose group were found to have this tumor type at 2 and 3 sites (liver and lung; liver, lung, and mesenteric lymph node, respectively).

The hypothesis that increased incidence of hemangiosarcoma observed at the mid dose level (3.9 mg/kg/day in males, 6.2 mg/kg/day in females) vs the high dose level (12.0 mg/kg/day in males, 16.2 mg/kg/day in females) may be based upon a decreased body weight in rats at the high dose is debatable. The rats in this study were not fed a calorie-restricted diet, nor was the amount of dietary intake strictly controlled. Although a statistically decreasing trend in mortality was observed for male rats, food intake, weight gain, and food efficiency were decreased at the 0.19 mg/ml dose (12.0 mg/kg/day in males, 16.2 mg/kg/day in females) in <u>both</u> sexes of rat. In addition, a review of the time to tumor formation for the rats observed with hemangiosarcoma at all dose levels shows that the tumors were observed at approximately the same time (from weeks 93-105), with only one rat at the mid dose observed with this tumor type at an earlier time point (week 66). It has been observed that in calorie-restricted animals, not only can tumor incidence be decreased, but the time to tumor can be delayed. It cannot be proved from the data in this study that such an effect occurred at the high dose.

c. <u>Non-neoplastic lesions and other findings</u>

At the high dose level, the following were observed:

Decreased body weight gain for weeks 1-13 (12% males, 16% females) and for weeks 1-105 (18% in males, 20% in females).

Decreased food consumption, food efficiency, and water consumption in male and female rats).

Increased incidence of liver masses (8/64 vs 4/64 in control) and fat vacuolation (11/32 vs 8/28 in control) in male rats.

Increased incidence of voluntary muscle wasting (9/64 vs 1/64 in control) in male rats

Increased incidence of microscopic abnormalities of the nasal cavity, voluntary muscle, and sciatic nerve, as well as decreased incidence of mineralization of the aorta in male and/or female rats.

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

Further details of these changes can be seen in the data evaluation record for this study, as the changes are too numerous to reproduce here.

The high dose of 0.19 mg/ml (12.0 mg/kg/day in males and 16.2 mg/kg/day in females) was considered adequate for testing of the carcinogenic potential of metam sodium in rats, based on the decreases in body weight gain, food efficiency, and macroscopic and microscopic pathology observed in both sexes in this study. The chronic portion of the rat chronic toxicity/carcinogenicity study was considered adequate by the Health Effects Division RfD/Peer Review Committee. This study was classified as core **minimum** data.

E. Additional Toxicology Data

1. Metabolism

Reference: Hawkins, D.R., Elsom, L.F., and Girkin, G. (1987): The Biokinetics and Metabolism of ^{14}C-Metam Sodium in the Rat. Study conducted by Huntington Research Centre, Cambridgeshire, UK and submitted under MRID # 406410-00.

Single oral doses of 10 mg/kg and 100 mg/kg ^{14}C-Metam sodium (purity > 99%) were administered to groups of male and female Sprague-Dawley rats (no. rats per group not specified). Urine and feces were collected up to 168 hours post-dose, while expired air was collected up to 72 hours post-dose. The time course of radioactivity in plasma was also investigated at the 10 and 100 mg/kg dose levels in five rats/sex/dose.

At the 10 mg/kg dose, urine was the major route of excretion, representing between 52-58% of the administered dose. Excretion through expired air represented between 32-38% of the administered dose, while between 3-4% was excreted through feces. At the 100

mg/kg dose, urinary excretion was decreased to between 37-42% of the administered dose, while excretion through expired air increased to between 47-53% of the administered dose. Fecal excretion remained low (between 1.5-1.8% of the administered dose).

At the low dose, the majority of collected radioactivity in expired air represented CO_2 (18-19% of administered dose) or COS and/or CS_2 (14-18% of the administered dose). A minor amount of MITC was observed (0.45-1.26% of the administered dose). At the high dose, the majority of collected radioactivity in expired air represented MITC (24-24.5% of the administered dose) and COS and/or CS_2 (18-21% of the administered dose), with only minor amounts of CO_2 (5.5-7.2% of the administered dose).

The time course of excretion was similar at the 10 and 100 mg/kg dose, with the shift being primarily in the percentages excreted through urine and expired air. A shift in biotransformation is indicated at the 100 mg/kg dose.

Tissue distribution at 168 hours post-dose showed the highest amounts of radioactivity in the thyroid (1.28-3.09 μg/g at 10 mg/kg; 6.24-7.55 μg/g at 100 mg/kg). Relative to other tissues, high concentrations of residual radioactivity were also observed in the liver, lung, and kidney. In general, tissue levels were higher in female rats than male rats at 168 hours.

Results of plasma time course measurements showed a T_{max} of 1.0 hours at the 10 mg/kg dose in both sexes, and a T_{max} of 0.25-1.0 hours at the 100 mg/kg dose. Half-life of elimination was unaffected by the increase in dose (60.8-74.1 hours at the 10 mg/kg dose [males and females]; 61.7-64.2 hours at 100 mg/kg [males and females]), and AUC was proportional to dose, indicating first-order kinetics at both dose levels.

The urinary and tissue profile of metam sodium metabolites was similar to that observed following dazomet administration; that is, the N-acetylcysteine conjugate of methyl isothiocyanate (MITC) was identified in urine as a major component (16.1-23.3% of the administered dose) at both dose levels, as was the glycine conjugate of MITC (5.1-8.2% of the administered dose at both dose levels). In the liver and kidney, the N-acetylcysteine conjugate of MITC was also identified as the major metabolite, as was the case for dazomet.

2. Mutagenicity

i.) Reference: Cifone, M.A. (1987): Mutagenicity Test on Metam Sodium in the Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay. Study # HLA 9736-0-447. Study performed by Hazelton Laboratories America, Inc. and submitted under MRID # 403056-01.

In this study, freshly isolated hepatocytes from male Fischer 344 rat liver were incubated with metam sodium at concentrations of 0.5, 1.0, 2.5, 5.0, 10.0, 50.0, 100.0, and 250.0 nl/ml. Incubations were at 37^{o} C for 18-20 hours in the presence of tritiated thymidine. The 250 nl/ml dose was selected based on the results of preliminary testing showing a relative survival range of 17% at 100 nl/ml to 55.3% at 50 nl/ml. Results of the main study showed that metam sodium caused no significant changes in nuclear labeling of primary rat hepatocytes at the concentrations tested.

Classification: **acceptable** (HED document # 006570)

ii) Hoorn, A.J. (1987): Mutagenicity Test on Metam Sodium in the Rec⁻ Assay with Bacillus subtilis. Study # HBC E-9642-0-404. Study performed by Hazelton Biotechnologies Veenedal Lab, Netherlands, and submitted under MRID # 403056-02.

In this study, Bacillus subtilis strains H17 and M45 were incubated in the absence and presence of metabolic activation (rat liver S-9) with metam sodium at doses of 0.1, 1.0, 5.0, 10.0, 25.0, 50.0, 100.0, and 150.0 µl/plate. Metam sodium failed to induce differential toxicity in Bacillus subtilis strains H17 and M45 at the concentrations tested.

Classification: **acceptable** (HED document # 007027)

iii) Engelhardt, G. (1987): Report on the Study of Metam Sodium in the Ames Test. Study # BASF 87/0208. Study performed by BASF Aktiengeselleschaft Dept. of Toxicology, FRG and submitted under MRID # 403056-03.

In this study, metam sodium was non-mutagenic to Ames Salmonella typhimurium strains TA92, TA98, TA100, TA1535, TA1537, and TA1538 in the absence or presence of metabolic activation (rat liver S-9). Concentrations tested were: 20, 100, 500, 1000, 1500, 2000, and 2500 µg/plate in the standard plate test, and 4, 20, 100, 200, 300, 400, 500, 1000, and 2500 µg/plate, in the pre- incubation test.

Classification: **acceptable** (HED document # 006570)

iv) Gelbke, H.P. (1987): In Vitro Cytogenetic Investigation in Human Lymphocytes with Metam Sodium. Study # BASF 87/0116. Study Performed by BASF Aktiengeselleschaft Dept. of Toxicology, FRG and submitted under MRID # 403056-04.

In this study, 48-hour cultures of human lymphocytes were exposed to 1, 5, 10, and 20 μg/ml metam sodium in the absence of metabolic activation and to 10, 20, and 40 μg/ml metam sodium in the presence of rat liver S-9. Incubations were for 24 hours at 37° C. In the absence and presence of metabolic activation, metam sodium demonstrated a dose-dependent and statistically significant increase in the number of chromosomally damaged cells. There was no significant increase in the numerical chromosome aberrations in treated vs solvent controls.

Classification: **acceptable** (HED document # 006570)

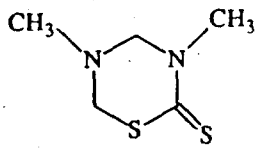
v) Gelbke, H.P. and Engelhardt, G. (1987): Cytogenetic Study in Vivo of Metam Sodium in Chinese Hamsters, Bone Marrow Chromosome Analysis. Study # BASF 87/0238. Study performed by BASF Aktiengeselleschaft Dept. of Toxicology, FRG and submitted under MRID # 403056-05.

Metam Sodium was tested for clastogenicity in Chinese hamsters after single oral doses of 150, 300, and 600 mg/kg. Five animals per sex were sacrificed at 6, 24, and 48 hours post-dose for examination of bone marrow cells. At the dose levels tested, metam sodium was not positive in Chinese hamster bone marrow.

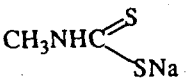
Classification: **acceptable** (HED document # 007027)

3. Structure-Activity Considerations

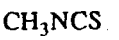
Although not structurally related, metam sodium and dazomet are related by virtue of their metabolism to methylisothiocyanate (MITC) or conjugates of MITC. Thiram and ziram can be considered structurally related to metam sodium as a dithiocarbamate. Structures of these chemicals are as follows:



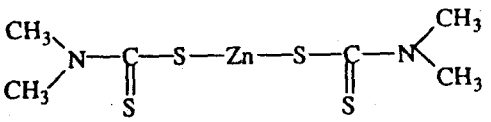
Dazomet



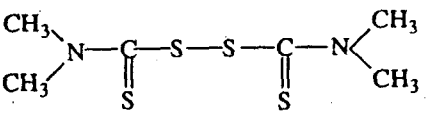
Metam Sodium



Methyl isothiocyanate (MITC)



Ziram



Thiram

Thiram

In a 2-year feeding study in rats with thiram (MRID # 421576-01), statistically significant positive trends were observed for development of thyroid C-cell adenomas in both male and female rats using dose levels of 0, 30, 150, and 300 ppm thiram. In addition, significant positive trends were observed for development of hepatocellular adenomas in both sexes, but the incidence at the 300 ppm dose was not statistically different from control incidence.

Data from IARC (Vol.12, 1976) list both the rat study mentioned above as well as human data derived from 105 Soviet workers making thiram for 3 years. These human data showed an incidence of 1 thyroid malignancy over the 3 year period for these 105 workers. A rat study in which both thiram and sodium nitrite were administered resulted in a high incidence of nasal adenomas, adenocarcinomas, olfactory carcinomas, and squamous cell carcinomas. No tumorigenic activity was observed from administration of thiram or sodium nitrite alone.

Based on these data, the conclusion of IARC with regard to thiram was that there was inadequate evidence of carcinogenicity. Thiram was assigned to risk category 3 (not classifiable).

MITC

Drinking water carcinogenicity studies have been performed for this chemical in both rats and mice, and these data have been submitted to HED for review (Accession #'s 257766 and 257759-257763). Although both studies were core graded as supplementary data, administration of MITC in drinking water did not appear to result in increased incidence of tumors in either rats or mice. However, the dose levels tested were not considered adequate for evaluation of the carcinogenic potential of MITC. The results of these studies have also been published in *Nippon Noyaku Gakki Shi* 15(2): 297-304 (1990), which concluded that no carcinogenic activity was observed for MITC in drinking water studies with rats and mice. It appears that a final determination of the carcinogenic potential of this chemical has not been reached.

Dazomet

Dazomet has been subject to peer review through the Health Effects Division Carcinogenicity Peer Review Committee. This chemical was classified as Group D - not classifiable as to human carcinogenicity. This decision was based upon both rat and mouse studies submitted to and reviewed by Toxicology Branch II. In the submitted mouse study (MRID # 418651-01), female mice were found to have a significant dose-related trend in hepatocellular adenoma and combined adenoma/carcinoma. No significant pair-wise comparisons with control were evident, and the prevalence of this tumor type in the $B_6C_3F_1$ mouse made it difficult to assign the tumors as a treatment-related effect.

In the submitted rat study (MRID # 418650-01), there appeared to be no treatment-related effect on the incidence of tumors in male and female rats.

Ziram

No carcinogenicity data have been submitted to the Agency for review. In an IARC monograph (Vol. 53, pages 423-438, 1991) it was stated that no human data are available for ziram. Studies have been performed in B6C3F1 mice and Fischer 344 rats. In mice, an increased incidence of benign alveolar and bronchiolar tumors was observed in female mice. In rats, a dose-related increase in the incidence of C-cell carcinomas of the thyroid were observed in male rats. The IARC conclusion for ziram was that of limited evidence of carcinogenicity. Ziram was not classifiable as to human carcinogenic risk.

Carbon disulfide

Carbon disulfide (CS_2) is one of the primary metabolic products of metam sodium after administration of a low (10 mg/kg) or high (100 mg/kg) oral dose (see pages 16-17 of this memo). In the July 1994 update on the Toxicological Profile for carbon disulfide (U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry), it was stated that there is no definitive evidence for an increased cancer potential from carbon disulfide in humans. Although an increased odds ratio for lymphocytic leukemia in rubber workers exposed to different kinds of solvents including CS_2 was reported, the large number of solvents used and design of the study preclude definitive association of CS_2 exposure with tumor development. In experimental animals, exposure of the A/J strain of mouse (females) to 300 ppm CS_2 for 6 hours/day, 5 days/week for 6 months resulted in a slight but statistically significant increase in the number of pulmonary adenomas per mouse and the number of tumors per tumor-bearing mouse lung (Van Stee et al., *J. Toxicol. Env. Health*, 17: 311-322, 1986). There are no chronic toxicity or carcinogenicity animal studies in the Agency's toxicology database for carbon disulfide.

4. Additional Information

On October 15, 1991, the Health Effects Division Developmental and Reproductive Toxicity Peer Review Committee (DRTPRC) met to discuss the existing developmental and reproductive toxicity database for metam sodium. At that time, only two studies had been submitted for review: A developmental toxicity study in rats (MRID #'s 41577101, 42170101, and 92097012) and a developmental toxicity study in rabbits (MRID # 40330901 and 92097013). Several deficiencies were observed in the review of these 2 studies, but the available evidence suggested that metam sodium induced developmental toxicity. In the rat study, treatment related effects (increased incidence of skeletal variations, retardations, and anomalies) were observed at doses as low as 4.2 mg/kg/day (LDT). In addition, two fetuses from one litter at the high dose of 51 mg/kg/day were observed with meningocoele. In this study, the maternal NOEL was stated as 4.2 mg/kg/day and the developmental NOEL as $\leq$ 4.2 mg/kg/day. In the rabbit study, the maternal NOEL was established at 12.6 mg/kg/day based on reduced body weight gain, while the developmental NOEL was established at 4.2 mg/kg/day, based on an increased number of dead implantations, reduced number of fetuses, and increased post-implantation loss at the mid and high dose. At the high dose, one fetus was observed with meningocoele, while

spina bifida was observed in one fetus at the high dose.

Two developmental toxicity studies and one 2-generation reproduction study have since been submitted and reviewed by Toxicology Branch II, Health Effects Division. These recently submitted data on developmental and reproductive toxicity of metam sodium support the earlier conclusion of the DRTPRC that metam sodium has developmental toxicity. The NOELs and LELs established in the new studies confirm the levels at which developmental toxicity was observed in the previous studies. It is noted that in the recently submitted rat study, meningocoele was observed at the high dose in one fetus from one litter, as was internal hydrocephaly in three fetuses from three litters, supporting earlier findings of neural tube defects / brain malformations from metam sodium administration at high doses.

F. Weight-of-Evidence Considerations:

The Health Effects Division Carcinogenicity Peer Review Committee considered the following toxicology data in determining the carcinogenic potential of Metam Sodium:

1. In the mouse carcinogenicity study, administration of metam sodium technical in drinking water for 2 years was associated with an increase in the incidence of angiosarcoma of the liver, spleen, and subcutaneous tissue. In male mice, there was a statistically significant increase in angiosarcomas of the liver and spleen (the most relevant sites) at the HDT. There was also a statistically significant positive trend for these tumors at both sites. In female mice there was a statistically significant increase in angiosarcomas of the spleen at the HDT; in the liver the increase was borderline (p=.055) at the HDT. There was also a statistically significant positive trend for these tumors at both sites. Angiosarcoma was considered a factor contributory to death in those mice with this tumor type at the HDT. A transitional cell papilloma in a single high dose male mouse and a transitional cell carcinoma in a single high dose female mouse, were also observed. According to the report, there was no treatment-related change in tumor latency.

2. In the rat carcinogenicity study, administration of metam sodium technical in the drinking water for 2 years was associated with a significant increase in the incidence of hemangiosarcoma in male rats at 1.3 and 3.9 mg/kg/day, but not at the high dose of 12.0 mg/kg/day. Tumor latency was similar between the low dose and mid dose groups.

3. In an *in vitro* cytogenetics assay with cultures of human lymphocytes, metam sodium demonstrated a dose-dependent and statistically significant increase in the number of chromosomally damaged cells, both with and without metabolic activation. This provided support for a mutagenicity concern. There was no significant increase in the numerical chromosome aberrations in treated vs solvent controls. Mutagenicity testing of metam sodium in an unscheduled DNA synthesis assay with primary rat hepatocytes, a rec assay with Bacillus subtilis, a Salmonella assay, and an *in vivo* cytogenetics assay in Chinese hamsters produced negative results in all these studies.

4. Metam sodium and dazomet are related by virtue of their formation of MITC or conjugates of MITC as the common end-product from metabolism of these chemicals. Thiram and ziram can be considered structurally related to metam sodium as a dithiocarbamate. The experimental evidence from these chemicals is limited, but indicates some evidence for carcinogenic potential. Carbon disulfide, another metabolite of metam sodium, also has some evidence of carcinogenicity.

5. Carcinogenicity in animals -- Metam Sodium

After a full evaluation of all of the data and supporting information regarding animal carcinogenicity, the Committee concludes that exposure to metam sodium resulted in an increased incidence of malignant angiosarcomas in both sexes of the CD-1 mouse. These tumors were considered a factor contributing to death in mice with this tumor at the high dose. Similar tumors (malignant hemangiosarcomas) in the male Wistar Tox rat were observed and provide further support for the induction of tumors by metam sodium. Data from mutagenicity studies and structurally related analogs add to the carcinogenicity concern for metam sodium.

The relevance of the tumor data to an evaluation of metam sodium's potential for human carcinogenicity is discussed elsewhere in this document.

G. Classification of Carcinogenic Potential:

The Peer Review Committee considered the criteria contained in the EPA's "Guidelines for Carcinogen Risk Assessment" [FR51: 33992-34003, 1986] for classifying the weight of evidence for carcinogenicity.

The Peer Review Committee agreed that metam sodium should be classified as a Group B2 - probable human carcinogen, based on increases in malignant angiosarcomas, by both pair-wise and trend analysis, in both sexes of the mouse, at doses that were adequate to assess carcinogenicity. The angiosarcomas were also a contributing factor to death. Additional evidence included the finding of similar tumors in the male rat (malignant hemangiosarcomas) which were considered to be supportive of the tumors seen in the mouse, as well as some evidence of mutagenicity and limited evidence from structurally related analogs.

The CPRC recommended that for the purpose of risk characterization, a low dose extrapolation model be applied to the animal data for the quantification of human risk (Q_1^*), based on the total incidence of angiosarcomas in male mice, at all sites combined. The CPRC also recommended that the registrant perform a dominant lethal study for germ cell effects to follow up on the positive mutagenicity evidence.