CASWELL FILE
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Data Evaluation Record

Study Title: Methylisothiocynate: A chronic oral (drinking water) toxicity and carcinogenicity study in the Rat

Study Type: Chronic/oncogenicity

Accession No.: 257766 Caswell No.: 573

Sponsor/Contracting Lab.: Nor-Am (Schering AG)/Hazelton Lab. Europe, Ltd

Report Date/Submitted: February 1984/April 26, 1985

Test Material: Methylisothiocynate (MITC), Technical 95.4-96.1%

Test Animal: Sprague-Dawley, CD strain rat [Charles River (UK)Ltd]

Test Doses: 0, 2, 10 and 50 ppm in drinking water

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Classification and Conclusions: This study is classified as Core Supplementary Data. The histopathological data must be provided in a summary form which includes the actual number of tissues examined/sex/group, not just the number of animals so examined. Also see items 2, 6, 7, 8, 9, 10 and 11, under "Methods" on page 2. Furthermore, it does not appear that a maximum tolerated dose was used in the study, unless the requested histopathological data provide this evidence. The weak, non-dose-related effect seen on body weight of males is judged to be insufficient evidence of a compound-related toxic effect, and no other effects were seen.

Methods: The methods from the report have been copied and are appended. Comments on the methods and reporting include the following:

1) The tables summarizing histopathological data did not indicate the number of each kind of tissue examined per group per sex; only the numbers of animals examined were given. In the presentation of individual results, missing tissues were indicated and should have been tabulated in the summary tables.

2) Liver, lungs and kidneys were not routinely examined histopathologically for animals in the low and middle levels. EPA Guidelines indicate that these tissues should be examined for all animals on study as an indication of their health status.

3) Baseline ophthalmological examination was not performed for some rats until the first few days of treatment had begun.

4) Test material was not given to animals that were isolated due to injury or illness.

5) The beginning body weight ranges for males were 136-216 g and for females were 82-196 g; these are unusually wide ranges.

6) The report did not provide a tabulation of clinical signs.

7) Individual food and water consumption data were not provided.

8) There was no summary of necropsy findings for rats that died during the second year or that were sacrificed terminally.

9) The test material was described both as a liquid and as a crystalline solid.

10) The report states that the plastic water bottles were modified to limit loss of MITC by volatilization. Yet, it is also stated that occasionally glass water bottles were used. It is important to know if all water bottles used were modified to preserve the concentrations of MITC and if not, to what extent unmodified bottles were used.

11) The analyzed concentrations of MITC presented to the rats for the first 22 weeks were not provided. Furthermore, the probable widest disparity between nominal and actual concentrations during the study was not given.

Quality Assurance: The report contained a list of audit dates (8 for the in-life phase, 1 for the final report and 1 for the revised report) and was signed by the Quality Assurance Manager.

Results:

A. Test Material - The rat study was conducted with two batches of MITC, batch number 28.166 and 29.482. The percentage purity for five analyzed samples (one of the former batch and four of the latter) ranged from 95.41 to 96.06% MITC. Although the material was described as "a light brown crystalline solid",

another description included in the report and stated to be from previous toxicological studies gave the appearance as a "colorless, yellow brownish liquid". This disparity in physical appearance should be explained. Can it be simply a matter of ambient temperature?

The test material was administered in the drinking water in plastic water bottles specially modified to limit loss of MITC by volatilization. The report states that occasionally glass water bottles were used. It is pertinent to know if all water bottles used were modified to limit loss of MITC since it is acknowledged to be so volatile; i.e., the report states that "the loss of MITC from solutions kept in conventional drinking bottles was shown to be almost complete after 24 hours".

Appparently, there was a particular problem regarding stability with the first batch of stock solutions; these stock solutions were used from study initiation through week 9. No analytical results for this batch were reported but it was stated that they were "poor" and test solutions were prepared daily, rather than weekly, from week 5 to week 9, when a fresh sample of test material (new batch?) was analyzed. The first reported results of analyses of the stock solutions and test solutions in the animal drinking water bottles were for week 23 and subsequent results were given approximately each three months thereafter. Results for the water bottle analyzes are shown in Table 1 (a reproduction of Report Table 13, page B-43). Some of the results show marked differences between nominal and analyzed values, e.g. the 2 ppm level for week 36 was only about 8% of nominal and several others were as low as only 50-65% of nominal, particularly for the 2 and 10 ppm levels. These results are for the day of preparation and 2-3 days later; the water bottles were filled on Fridays, Mondays and Wednesdays from stocks prepared each Friday. It is not clear whether the table values were from fresh solutions made on Friday or from solutions dispensed on Mondays or Wednesdays. This can be an important difference, for reported results show that there even was deterioration of the frozen stock solutions stored in the dark. Furthermore, the table values are means of 6 individual bottles (3 male and 3 female) and there was wide variability among these individual results, e.g. the means of 50.0% and 54.2% of nominal reported for 3 days after preparation for 2 and 10 ppm solutions for week 75 had ranges of 16-59% and 24-78%, respectively.

The worst cases would have been the water bottle concentrations on Fridays before fresh solutions were dispensed that day. There was no indication that any of the reported results were from this time period. Consequently, probably the widest differences between nominal and actual concentrations are not known.

As the table indicates, analysis of control solutions for weeks 23 and 36 suggested contamination with MITC, but the

Table 1- Compound analyses in drinking water.

TABLE 11

Summary of MITC analysis on samples taken from drinking bottles

Week	Group and nominal MITC concentration											
	1 0 ppm			2 2 ppm			3 10 ppm			4 50 ppm		
	On day of preparation Mean ppm	%	3 days after preparation Mean ppm	%	On day of preparation Mean ppm	%	3 days after preparation Mean ppm	%	On day of preparation Mean ppm	%	3 days after preparation Mean ppm	%
23*	0.30	-	0.20	-	1.52	75.3	1.22	60.0	7.90	79.0	7.35	73.5	40.13	80.2	34.11	68.3
36	0.10	-	0.13	-	0.17	7.67	0.18	8.17	9.13	91.3	8.60	86.0	45.3	90.5	40.9	81.8
50*	-	-	-	-	1.90	95.2	1.72	86.2	9.23	92.3	8.23	82.3	44.05	88.0	43.9	87.8
62	-	-	-	-	2.07	103.1	1.60	80.0	10.86	108.6	9.85	98.5	53.77	107.6	50.55	101.0
75	-	-	-	-	1.60	80.2	1.02	50.0	9.02	90.2	5.42	54.2	42.52	85.0	39.93	79.8
88	-	-	-	-	1.58	79.7	1.38	68.7	8.7	87.0	6.4	64.0	41.02	82.0	35.50	71.0
101	-	-	-	-	1.21	61.3	0.98	49.8	7.33	73.3	6.05	60.5	37.35	74.8	35.28	70.7
Overall mean	-	-	-	-	1.45	71.8	1.15	57.6	8.88	88.8	7.41	74.1	43.45	86.87	40.02	80.1

* Samples taken on day of preparation and 2 days after preparation.

investigators stated these results were due to an impurity in a solvent and after the analytical procedure was modified there was no further indication of contamination of the control water.

In conclusion, the analyzed dose levels presented the rats for the first 22 weeks of the study were not provided. Furthermore, the widest disparity between nominal and actual concentrations was not defined. Since there is an acknowledged problem with maintaining the concentration of MITC during dosing because of the high vapor pressure, assurance that the rats received the intended concentrations is particularly critical for this study.

B. Survival - Survival was good in this study, with the following percentages of rats alive at 18 months: 94, 92, 90 and 86 for males and 90, 80, 97 and 95 for females of the 0, 2, 10 and 50 ppm groups, respectively; however, by termination at 105 weeks the percentages alive had dropped precipitously to 65, 63, 58 and 64 for males and 43, 33, 53 and 53 for females for the same groups, respectively. Survival did not appear to be affected by MITC treatment.

C. General Observations: The investigators did not present a tabulation of clinical signs but stated that the incidence and severity of the following most frequently observed signs did not appear to be affected by treatment: epilation, tooth malocclusions with associated soft tissue damage, staining around the eyes and of fur generally, staining or swelling around the genitals and pilorection in females.

D. Body Weight: The spread of body weights at study initiation was very wide, being 81-216 grams. The groups were adjusted so that each female group had a mean of 162 grams and the male groups averaged 185, 186, 185 and 179 grams for the 0, 2, 10 and 50 ppm groups, respectively.

The high level males consistently weighed less than controls, as did the low level, also, after week 8, but the low level weight was not as depressed as that of the high level. The mid level males had lower body weights than controls beginning about week 30, but the effect was less than for the other two male groups. All male groups, including controls, had an unexplained loss of about 20 grams at week 22, with recovery within two weeks. At termination, weights of the treated males were 92, 97 and 91% of the control for the 2, 10 and 50 ppm groups, respectively.

Female bodyweight was not adversely affected by treatment with MITC. The 2 ppm group weighed more than controls beginning early (3rd week) in the study, as did the 10 ppm group beginning about week 50. The highest level females also weighed more than controls during the second year of the study.

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Group mean body weights for 2 ppm males were checked and had been correctly tabulated from individual data to report table 2.

E. Food Consumption: Treated males had similar food consumption to controls. All male groups showed a decrease in food consumption for week 22; this ranged from a 6 gram mean/rat difference for the low level to only a 1 gram mean/rat difference for the high level.

Food consumption was similar among all groups of females also. This sex did not show the obvious decrease for week 22 seen for the males.

Individual food consumption data were not provided.

F. Water Consumption: The high level group generally showed a lower water intake than the controls; this was seen more consistently for males than for females. Lower levels occasionally drank less than controls but the 2 ppm females ocasssionally drank more than controls. Consequently, the only group showing a consistent depressed water intake was the 50 ppm group.

Individual water consumption data were not provided.

G. Compound Intake: The report stated that "the majority of the study was conducted with doses of 0.08, 0.37 and 1.60 mg/kg and 0.12, 0.56 and 2.65 mg/kg for males and females" of the low, mid and high levels, respectively and that these doses were calculated from week 26 forward.

On two occasions, rats isolated because of injury or illness were not given MITC in their water; the dates and rats were not identified.

H. Ophthalmology: Ophthalmic findings occurred with similar frequencies for control and treated rats. The total numbers affected were 10 vs 11 males and 8 vs 7 females for control and high levels, respectively. Keratitis, lenticular opacity and hyperreflective retina were the most frequent findings.

Data for Table 6, "Summary of Ocular Abnormalities", were correctly tabulated from the individual data.

I. Hematology: Hematological parameters were not affected in either sex at any level by dosing with MITC.

Mean hematological data of males for week 52 were correctly tabulated from the individual data to the summary table (No. 7).

J. Clinical Chemistry: Treatment with MITC did not affect clinical chemistry values of either sex at any level. One high level male (#199) at termination had an unusually high alkaline phosphatase activity (3198 I.U./l compared to a mean value of

181 I.U./l for control males terminally). This extremely high value caused the mean for the group to be over two-fold that of control males, but other individual values were within the range of the controls.

Mean clinical chemistry data of females for week 103 were correctly tabulated in report Table 8 from the individual data.

K. Urinalyses: Urinary volume for treated high level males was noticably less than for controls for weeks 13 and 78, and for treated females the volume was less for weeks 26 and 52; yet these differences were not reflected in specific gravity values. Treated males had 2 plus urinary blood for weeks 78 and 103 compared to 1 plus for controls but these findings were not reflected by increased erythrocytes noted during microscopic examination nor were they accompanied by presence of casts.

Treatment with MITC did not appear to adversely affect urinary parameters.

L. Organ Weights: There were no indications of a compound-related effect in brain, liver, heart, gonad, thyroid or kidney weights. For both the adrenals and pituitary, the presence of tumors in these tissues precluded the use of organ weight data as an indication of compound toxicity because of the increased degree of weight variability. Likewise, the presence or absence of a thymus was so variable among the groups that these organ weight data were not useful in detecting discrete changes from control data.

Mean terminal organ weights for high level males were correctly tabulated in report Table 11 from the individual data.

M. Macroscopic Pathology: The report presented a summary of macroscopic pathology only for rats of the interim sacrifice at 52 weeks and a separate summary for those rats which died during the first 52 weeks of the study; there was no summary for those rats which died during the second year or that were sacrificed at study termination. A summary is required for all rats in the study and the relationship between macro- and micropathology should be discussed.

Necropsy findings for rats of the interim kill (5 of each sex from the control and high level groups) and for those dying during the first year were not remarkable nor did the findings appear to be compound-related. The highest incidences were for pulmonary findings and these were distributed similarly among all groups. Three pituitary masses and one subcutaneous mass were found in rats that died in the low level and a pituitary mass was found in the sacrificed controls.

Ataxia/lateral deviation and depression/lethargy were each listed for 2 female 2 ppm rats. These findings were listed as

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macroscopic observations but they are more commonly grouped as clinical observations rather than as necropsy findings.

N. Histopathology: The report did not summarize the histopathology results on the basis of the number of tissues examined per type/sex/group. The summary tables gave only the total number of animals examined per sex and group, yet in the tabulation of individual data there were notations of missing tissues. Consequently the needed figures could have been compiled from the individual data submitted, but this is a lengthy process that should have been done routinely as part of the report preparation. A definitive assessment of the histopathological results must await review of the data presented in the proper summary form. Therefore, the following discussion of the results must be regarded as preliminary and final conclusions will be made after receipt of proper summaries.

The lungs, liver and kidneys of all animals in the low and middle dose levels were not routinely examined. The EPA Guidelines suggest that these tissues be examined in all rats as an indication of the health status of the animals.

1) Non-neoplastic lesions: The animals showed relatively high incidences of eosinophilic cell alterations and angiectasis of the adrenals (females), Harderian gland adenitis (females), glomerulonephritis (both sexes), myocarditis/myocardial fibrosis/scarring (both sexes), and hepatic vacuolation (females). The incidences for these lesions and for others for which the incidence was increased are shown in the following Table 2 (data taken from report Table 6, Appendix 14.2). For those lesions showing an increased incidence in treated rats there usually was no dose-response relationship and usually only one sex showed the increased incidence. Furthermore, in some of these cases, the controls of the unaffected sex had an equal or higher incidence. Consequently, due to these circumstances, no target organ was identified and administration of MITC does not appear to have influenced the incidence of non-neoplastic lesions in rats of this study.

2) Neoplastic Lesions: Neoplastic lesions which occurred at the highest frequencies are listed in the following Table 3 (data taken from report Table 3, Appendix 14.2).

The most frequent tumors were those of the female mammary gland where the total incidence for benign and malignant tumors were 32, 35, 41 and 36 for 0, 2, 10 and 50 ppm groups, respectively. Females of treated groups had slightly increased incidences of multiple benign tumors (11, 15, 18 and 18) but lower incidences of single benign tumors (20, 15, 17 and 16 for the 0, 2, 10 and 50 ppm groups, respectively), than controls.

There was an unexpected incidence of brain tumors in males; the totals for glial tumors were 1, 2, 4 and 1 for the 0, 2,

TABLE 2 - INCIDENCE OF NON-NEOPLASTIC LESIONS

(Data taken from Report Table 6, Appendix 14.2)

ppm	0		2		10		50	
	M	F	M	F	M	F	M	F
No. of Animals Examined	60	60	55	59	55	60	60	60
Adrenals-Eosinophilic Cell Alteration	6	27	2	22	5	13	10	27
Angiectasis	0	16	0	10	1	8	0	20
Harderian Gland-Adenitis	13	21	4	16	4	11	14	24
Heart-Myocarditis/ Myocardial Fibrosis/ Scarring	30	26	6	10	12	6	35	23
Kidney-Pelvic Microcalculi	0	14	1	15	1	5	1	21
-Renal Scarring	0	2	0	1	0	0	7	0
-Glomerulonephritis	30	26	7	9	7	12	29	20
Liver-Fatty Vacuolation/ Vacuolation	9	17	9	21	2	15	14	23
Biliary Proliferation/ Sclerosis	11	5	3	5	4	6	13	10
Lungs-Calcification/Mineralization of Pulmonary Artery	14	8	6	12	8	5	16	15
Perivascular WBC Infiltration	9	4	5	9	5	7	13	5
Ovaries - Cysts	-	6	-	6	-	9	-	13
Spleen-Hyperplasia/Inc. Hematopoiesis/Lymphopoiesis	8	3	5	6	2	5	5	11

TABLE 3 - TUMOR INCIDENCE - ALL ANIMALS

(Data taken from Report Table 3, Appendix 14.2)

ppm	0		2		10		50	
	M	F	M	F	M	F	M	F
# of rats examined	60	60	55	59	55	60	60	60
Brain								
Glioblastoma					1	0		
Mixed Glioma	1	0	0	1	1	0		
Astrocytoma					1	0	1	0
Oligodendroglioma			2	0	1	0		
Total Glial Tumors	1	0	2	1	4	0	1	0
Fibrous Memingoma							1	0
Histiocytic Memingoma							1	0
Total Memingoma Tumors	0	0	0	0	0	0	2	0
Mammary Gland								
Benign - single	2	20	0	15	1	17	0	16
Benign - multiple	0	11	0	15	0	18	0	18
Malignant - single	0	1	0	3	1	1	0	0
Malig. - single & benign			0	1	0	5	0	2
Malig. - multi & benign			0	1				
Total benign & malignant	2	32	0	35	2	41	0	36
Pancreas								
Islet Cell Carcinoma	1	0			3	0	1	1
Islet Cell Adenoma	2	0	0	1			5	2
Islet Cell Adenoma and Carcinoma	1	0						
Total Islet Cell Tumors	4	0	0	1	3	0	6	3
Exocrine Adenoma	4	0	0	0	0	0	0	0
Exocrine Adenocarcinoma	2	0	0	0	0	0	0	0
Total Exocrine Tumors	6	0	0	0	0	0	0	0
Pituitary								
Adenoma	19	44	15	39	12	41	20	38
Adenoma/carcinoma			0	1			0	2
Total Tumors	19	44	15	40	12	41	20	40
Subcutaneous Tissue								
Fibroma- single	3	2	3	0	2	6	8	3
Fibrosarcoma	2	0	3	0	3	2	2	2
Lipoma	2	3	2	2	4	1	2	2
Histiocytic sarcoma	1	1	1	0	2	0	0	1
Cutaneous histiocytoma			1	0				
Sarcoma-unspecified/ undifferentiated/anaplastic			1	0	2	0		
Hibernoma			1	0				
Hemoangiosarcoma							1	0

CONTINUED Table 3

ppm	0		2		10		50	
	M	F	M	F	M	F	M	F
# of rats examined	60	60	55	59	55	60	60	60
Skin								
Papilloma/Squamous papilloma	2	0	4	1	7	0	2	0
Basal Cell Tumor			1	0	2	1	0	1
Baso-squamas Carcinoma	1	0						
Dermal Fibroma-single	2	0	1	0	7	0	2	0
Dermal Fibroma-multiple	1	0			1	0		
Thyroid								
C-Cell Adenoma	6	6	2	3	2	3	7	8
C-Cell Carcinoma	3	2	1	0	0	2	5	2
TOTAL	9	8	3	3	2	5	12	10
Follicular Adenocarcinoma/ carcinoma	0	1					4	0
Follicular Adenoma			1	0	1	1		
TOTAL	0	1	1	0	1	1	4	0
Uterus								
Endometrial Polyp		1		1		2		5
Adrenals								
Pheochromocytoma	9	4	2	1	2	0	7	1
Cortical Adenoma							0	1
Cortical Carcinoma					0	1		

10 and 50 ppm males, respectively. One female in the low dose group had a glial tumor and two males each of the high dose group had a meningoma.

Pituitary adenomas were prevalent in both sexes but females had over twice the incidence found in males; the incidences were 19-44, 15-39, 12-41 and 20-38 for males-females of the control, low, mid and high levels, respectively.

Thyroid tumors were also numerous in both sexes with the totals for C-cell adenomas/carcinomas being 9-8, 3-3, 2-5 and 12-10 for males-females of the 0, 2, 10 and 50 ppm groups, respectively. Males of the high level had 4 follicular carcinomas/adenocarcinomas compared to none in the controls.

Pheochromocytomas were also frequent; the numbers were 9-4, 2-1, 2-0 and 7-1 for males-females of the 0, 2, 10 and 50 ppm levels, resepctively.

As is obvious from the above discussed tumor incidence and from the table data, there is no indication for an increased tumor incidence due to treatment with MITC; however, final assessment will have to await review of the requested data summarizing the incidence on the basis of the number of tissues examined/sex/group.

Discussion: No compound-related or dose-related effects were seen in the rats of this study as a result of ingestion of MITC in their drinking water up to a level of 50 ppm; however, final assessment of histopathological data must await requested summarized data which are to include the actual number of tissues examined/sex/group.

This study does not appear to have included a maximum tolerated dose. Barring the possibility that the requested proper summary of histopathological data will indicate a compound-related effect, no clear-cut evidence for one has been seen in the data so far. Treated males showed slightly depressed body weights compared to controls, but the effect was not dose-related since the mid-level was the least affected and similar degrees of weight depression were seen for the low and high levels. Females did not show any depression of body weight; in fact, all levels of treated females weighed more than their controls.

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