8-5-86
CASWELL FILE

Data Evaluation Record

Study Title: Two-year Chronic Oral Toxicity and Oncogenicity Study with Methyl Isothiocyanate in Albino Mice (106 weeks final report) and Eight Separate Water Stability Studies with Methyl Isothiocyanate

Study Type: Chronic/Oncogenicity and Test Material Water Stability
Caswell No. 573, Accession No. 257763 and Nos. 257759-62

Sponsor/Contracting Laboratories: NOR-AM Chemical Co. (Schering AG)/ Nippon Experimental Medical Research Institute Co, Ltd., Japan

Report Date/Submitted: Dec. 1980/4-26-85

Test Material: Methyl Isothiocyanate, technical (MITC)
Lot # MS 25206, purity 93.14%, November 22, 1977;
Supplier: Shionogi & Co., Ltd.

Test Animal: ICR: JCL mice supplied by Clea, Japan
70 each males and females/group

Test Doses: 0, 5, 20, 80 and 200 ppm

Study Reviewed by: Winnie R. Teeters, Ph.D. W. Teeters
Section V, TOX. Branch/HED

Approved by: Laurence D. Chitlik, D.A.B.T. 3/5/86
Head, Section V
Toxicology Branch/HED

Classification and Conclusions: This study is classified as Core Supplementary Data. The actual number of tissues examined/sex/level must be provided. Furthermore, information on compound preparation and analytical data for the test compound in the water offered the mice are required.

Assessment of a possible histopathological compound effect must await receipt of requested data. The only compound effect established thus far is a slight depressant effect on body weight of the 80 and 200 ppm males and the 200 ppm females.

Methods: The methods from the study have been copied and appended. The following items were noted during review of the study.

1) The report failed to provide the actual number of each type of tissue examined histologically per group per sex; only the number of animals examined per group per sex was given.

2) Dosing was accomplished via the drinking water but analysis of the test compound in the water offered the mice was not

reported nor was there any discussion regarding compound preparation. However, there were eight separate stability studies of the test compound in water submitted with this study. This issue is further discussed more fully under the "Results" section in the subpart titled "Test Material".

3) Male mice weighed 14-23 grams at initiation of the study; this is an unusually wide range. Females weighed 15-19 grams.

4) There was no tabulation of daily clinical signs; there was only a summarizing statement on this subject for the entire duration of the study.

5) The following Guideline tissues were not examined: trachea, salivary glands, female mammary gland, esophagus, caecum, colon, rectum, spinal cord, gall bladder and aorta.

6) Some reporting "items" apparently resulted during translation, e.g. the report stated the "Four-week-old male and female ICR: JCL mice were purchased from Clea Japan, Inc. and were preliminarily bred for one week"; it is assumed that the mice were "held" for one week rather than bred for this period. There was also a statement that "The intake of the chemical was calculated based on the daily average intake of water, which was adjusted every day (the intake in a 24-hour period)"; it is assumed that "adjusted" means that the treated water was made up fresh daily, which is so stated in another part of the text.

Quality Assurance: There was a statement to the effect that the report had been audited by Nippon Medical Research Quality Assurance Unit and was considered to be an accurate presentation of the data produced during the course of the study.

Results:

A. Test Material - The test material was presented to the mice in their drinking water. The report stated that "MITC was mixed with drinking water every day to obtain definite concentrations based on the calculation of effective components. The water was given ad libitum from a glass bottle with a silicone stopper."

No analytical data of the solutions given to the mice were provided. Eight separate stability studies of MITC in water accompanied this oncogenicity study. They were all conducted at the Nippon Experimental Medical Research Institute Co., Ltd. and although dated 12-16-1980, represented analyses done about every three months over approximately the same two-year period as the mouse study; however, the first analyses were dated three months after initiation of the mouse study.

The stability studies were well documented and contained standard curves and chromatograms for the data. The studies consisted of analyses of water solutions of the same concentrations as used in the

animal study; the solutions were made up twice a day (morning and afternoon) and analyzed immediately and at twice daily intervals up to 3 days for studies 3-8 and up to 21 days for studies 1 and 2. In all studies, stability of 5, 20, 80 and 200 ppm concentrations was determined with storage in a glass animal water bottle and stability of the highest concentration was studied with storage in a commercially available polycarbonate animal water bottle (glass water bottles were used in the mouse study) and a glass volumetric flask. The first study also contained data on percent recovery and data comparing each of the two water bottles with and without an air space present above the liquid level, as would necessarily result during use of a bottle in an animal study.

The twenty-four hour data for the four concentrations in the glass water bottle (without an air space) showed that the concentrations were mostly within 90% of the mean concentration of the two analyses performed immediately after preparation. The glass water bottle was found to be superior to the polycarbonate one in maintaining the MITC concentration and the concentration was best maintained in the volumetric flask. For each type of water bottle, the presence of an air space above the liquid promoted the loss of MITC from the liquid.

The logic of continuing to investigate the same parameters after the first stability study, for a repetition of seven times, is incomprehensible, particularly in light of the fact that not one analysis was conducted to answer the critical point, that being the concentrations the mice received and, particularly, data on the concentrations in the water bottles just before they were replenished with fresh solutions. It is logical to expect that under the pressing needs of the study daily routine which required that fresh solutions be given 360 mice each day that the same care would not be given to preparing and maintaining the stability of the animal solutions as would be given, in the less hectic pace and environment of the analytical laboratory, for preparation and maintenance of the solutions scheduled for analyses.

Can an explanation for the apparent redundant stability studies and the critical lack of analytical data on the animal solutions, be that during translation important, critical information has been lost.

Pertinent to an evaluation of the animal study, however, and additional to the stability issue, is the fact that the animal study report, itself, does not contain any information on compound preparation; there is only the statement regarding daily presentation of fresh solutions to the animals.

B. Clinical Signs: There was no tabulation of periodic clinical signs in the report; there was only a summary statement in the text that no abnormal findings were seen in the 5 and 20 ppm groups and that equal numbers of each sex in the 80 and 200 ppm groups showed raised hair and a dull coat beginning 30 days into the study and lasting "a long time".

C. Mortality: Treatment with MITC did not appear to affect mortality. At 18 months, there had been only one death among males (200 ppm group) whereas 7, 6, 3, 10 and 4 females had died in the 0, 5, 20, 80 and 200 ppm groups, respectively. By study termination at 106 weeks, mortality among the groups was comparable, being 35, 25, 29, 28 and 37 for males and 37, 38, 37, 39 and 37 for females for 0, 5, 20, 80 and 200 ppm groups, respectively, which had contained 58 mice/group/sex initially (not including 6/sex/group, each sacrificed at 26 and 52 weeks).

D. Body Weight: After only the first week of dosing, males of the 200 ppm group showed a significantly ($p<.001$) lower body weight and after the second week, males of the 80 ppm group also had significantly ($p<0.01$) lower body weights than controls. This situation persisted with only a few exceptions through week 98. The 20 ppm males occasionally had lower weights; the differences from controls were consistently significant for weeks 84-98 and from week 96 to study termination (week 106) this group had the lowest weight of all male groups. Body weight gains at weeks 26 and 52 were significantly less than controls for the 80 and 200 ppm groups but overall gains (weeks 0-106) for none of the treated males were significantly different from their controls.

The only female group showing a similar effect to the males was the 200 ppm group. The lower body weight ($p<.01$) for this group was first seen at week two and occurred quite regularly (42/67 periods) thereafter. Lower level female groups occasionally had mean body weights that were greater than their control. Body weight gains at week 26 and 52 were significantly less than controls only for the 200 ppm females, but overall gains (week 0-106) for none of the treated females were significantly different from control.

Although body weight changes for 200 ppm males were significantly less than controls, even to the $p<0.001$ level, there was only one period, week 90, for which the weight difference amounted to 10% less, and for the other periods the differences were usually only 5-7% less than control. For females, also, the week 90 weight was the only period for the 200 ppm group for which the difference from control, although at the $p<0.01$ level, approached 10% less (actually 9.1%). For females, the differences were usually less than 5% lower than control. Consequently, the biological significance of the effect is unclear, yet it did occur in both sexes at the high level (200 ppm) rather consistently, particularly for males, and was seen persistently in the next lower level (80 ppm) of males, also.

E. Food Consumption and Food Efficiency: There were no significant differences between treated groups of either sex and their controls for food consumption or food efficiency.

F. Water Intake and MITC Dose: There appeared to be only small differences for mean water intake for either sex between treated groups and their controls. (These data were not analyzed statistically nor were any statistical measures of variability provided for them.)

Male mice of the 2 lower levels (5 and 20 ppm) generally consumed similar or slightly more water than controls but there appeared to be a dose-related lower water consumption for the 80 and 200 ppm males. A similar pattern was found for the females, with there being a dose-related lower water intake for the 80 and 200 ppm groups. Consequently, there was a general parallel between the depressant effect on water consumption and body weight, but no relationship seen between body weight and food consumption.

The overall average (male and female) compound intake for the entire study was 0.87, 3.48, 12.43 and 27.37 mg/kg/day for the 5, 20, 80 and 200 ppm groups, respectively (data taken from report table 12). As is usually the case, females had a slightly higher per weight intake than the males.

G. Ophthalmology: All mice were examined for ocular effects at 13, 26, 52 and 106 weeks. Findings appeared random with no relation to dose and included cataract, bulb atrophy, localized corneal and lens opacity and corneal keratitis. The total numbers with lesions were 2, 2, 3, 2, and 3 for males and 5, 2, 1, 1 and 5 for females of the 0, 5, 20, 80 and 200 ppm groups, respectively.

H. Hematology: The only notable hematological differences between treated groups and controls were the tendencies of both sexes of the 80 and 200 ppm groups to have a decreased erythrocyte count and an increased reticulocyte percentage and hemoglobin and hematocrit values tended to parallel the erythrocyte depression. These differences were significant only at the 52 week determination and only for males (RBC:$p<0.05$ for 80 and 200 ppm; hematocrit: $p<0.01$, 80 ppm; reticulocyte percentage: $p<0.05$, 200 ppm). At 26 weeks, the high level females had an increased platelet count, but the control appeared slightly low for the period. No differences from controls were noted for either sex of treated mice at any level at 106 weeks.

I. Clinical Chemistry: At 26 weeks, there were significant decreases for total protein ($p\leq 0.05$ for all) for males and females of the 80 and 200 ppm groups, for blood urea nitrogen ($p<0.01$) for males of these groups and for cholesterol ($p<0.05$) for females of the 200 ppm group. At later periods of 52 and 106 weeks there were not even tendencies for these effects.

Terminally (106 weeks), the 200 ppm females had slightly increased glutamic oxaloacetic transaminase activity (110.86 ± 16.04 and 138.38 ± 32.19 K.U. for controls and treated, respectively) and males of this level showed the same tendency. Both sexes of this level at 52 weeks and the females at 26 weeks had shown a similar tendency for increased activity. There was also a tendency for slightly increased glutamic pyruvic transaminase activity at early intervals (both sexes of the 200 ppm group at 52 weeks and females only at 26 weeks) but neither sex had elevated values at 106 weeks when the study was terminated.

The increased transaminase activity was minimal and the changes in total protein, blood urea nitrogen and cholesterol were temporary, occuring only at the first determination; furthermore, there was no histological or other evidence of toxicity which corroborate these findings. Consequently they are assumed to have very little biological significance.

J. Urinary Findings: There were no notable differences terminally between treated and control mice for any of the urinary parameters. Increases in sodium and potassium had been seen earlier. At 52 weeks, potassium increases were significant for 80 ppm males ($p<0.01$) and for 80 and 200 ppm females ($p<0.05$) and slightly increased but not significant for 200 ppm males and 20 ppm females, but these changes were not dose related. Slight sodium increases were noted for 200 ppm males at 26 and 52 weeks and there were dose-related slight increases for 80 and 200 ppm females at 52 weeks but none of the sodium increases was significant.

Although some of the electrolyte increases were significant, they were not dose related nor persistent and thus are of uncertain biological significance.

K. Organ Weights: Relative, but not absolute, brain weight was significantly ($p<0.01$) increased for 20 ppm males at termination; a similar but not significant, effect was seen also for 80 ppm males. In both cases this effect may be explained by the slight decrease in body weight of these groups compared to their control. At 26 weeks, males of the 20 ppm group had shown a significant ($p<0.05$) absolute brain weight increase without an accompanying increase in relative weight, but the change was an increase of only 6%.

Terminal male relative liver weights were significantly increased ($p<0.05$) for the 80 ppm group; there were also absolute increases for males of this group and the 200 ppm group, but neither attained significance. There were no significant changes for this organ in males at either interim sacrifice.

At 106 weeks, males of the 200 ppm group had an increased ($p<0.05$) relative urinary bladder weight; this group also had an increased absolute weight, as well, although not signficant, and the 80 ppm group had slightly increased, but not significant, both absolute and relative urinary bladder weights. Individual weights were checked for the highest levels and it appears that some of the bladders may have been weighed when they were distended by urine as 4/6 of this group showing markedly elevated weights also had a necropsy finding of "distended" but none had a reported histological finding to explain the increased weight.

At the 52 week interim sacrifice, males of the 80 and 200 ppm groups had several significant organ weight changes (e.g., absolute and relative weights of pituitary, thyroid and spleen were increased in the 200 ppm group and urinary bladder absolute, spleen relative and left thyroid absolute and relative were increased for the 80 ppm group) but for each of these an apparent explanation is provided by

the fact that the controls were unusually low; this judgement is based on observing that for several male organs the weights for controls were less than for control females of about 9 grams lower mean body weight sacrificed at this period, and urinary bladder weights for 52 week male controls were less than those of control males of similar weight sacrificed at 26 or 106 weeks.

Therefore, in summary, although there were several significant organ weight changes at interval or terminal sacrifices, either they appear to have logical explanations that are not related to compound administration, they are not dose-related or they appear to have no biological significance; furthermore, none is supported by evidence of histological alteration.

L. Necropsy: There were no dose-related necropsy findings; all findings appeared random among the groups.

In those instances among mice sacrificed at termination in which treated mice had a higher incidence than the control, the lowest dose had an equal or higher incidence than the highest group. As examples of this, ovarian cystoma was prevalent with an incidence of 6/21, 12/20, 9/21, 8/19 and 9/21, as was myomatous appearance of the uterus with an incidence of 5/21, 10/20, 3/21, 4/19 and 5/21 for the control, 5, 20, 80 and 200 ppm groups, respectively. Additional prevalent findings were pulmonary hyperemia, nodes and liver-like changes for lungs of both sexes; hepatic tumors, distention of the urinary bladder, testicular softening and enlargement of the seminal vesicles of males; swelling and turbidity (?) of the spleen and renal discoloration in both sexes; and uterine relaxation, ovarian hemotoma and mesenteric lymph node enlargement in females.

The most prevalent finding among animals that died was splenic enlargement among females, and although the incidence was higher in treated mice, again the lowest dose level had the highest incidence: 3/37, 14/38, 5/37, 6/39 and 9/37 for the control, 5, 20, 80 and 200 ppm groups, respectively.

M. Histopathology: The report did not contain a tabulation of the histopathological results as a ratio of the number of mice with positive findings to the number examined for that particular tissue per group per sex. The report only presented the total numbers of mice examined per group per sex. It is common to lose a few tissues to autolysis or other management factors in a long term study, particularly for a small animal such as the mouse. Tables listing the individual tissues examined by animal number were present but these only listed positive findings; therefore the crucial total number examined per tissue/sex/group could not be determined from the report.

The following EPA Guideline tissues were not routinely examined: trachea, salivary glands, female mammary gland, esophagus, caecum, colon, rectum, spinal cord, gall bladder and aorta.

1) Non-neoplastic: The data did not indicate a target organ. For those tissues of treated mice sacrificed at 106 weeks having

a higher incidence of lesions than in the controls, the group incidence appeared random, with no indication of a dose-relationship since often the highest incidence was in the lowest dose group. There did appear, however, to be a weak dose-related incidence of cystic ovaries: the incidence was 2/21, 4/20, 3/21, 5/19 and 10/21 for the control, 5, 20, 80 and 200 ppm groups, respectively. Yet, this is a common lesion and the dose-response was poor, at best. Uterine cysts were also prevalent but the incidence was similar among the controls and the 5 and 20 ppm groups, with a lower incidence in higher level groups.

The two most common findings were small cell infiltration of multiple organs and amyloid degeneration of multiple organs, both of which are to be expected in aging mice. The most prevalently affected organs for small cell infiltration were the liver, spleen, kidney, urinary bladder, stomach, pancreas, thymus, bone marrow and mesenteric lymph node and for amyloid degeneration the organs affected most often were the spleen, kidney, duodenum, small intestine, thyroid, adrenal, skin and ovaries.

2) Neoplastic: The most prevalent neoplastic findings were pulmonary adenocarcinomas of males and leukemia of females, but for both lesions the incidence was similar among control and treated mice. These incidences and those for other neoplasms for which there were multiple types of tumors in an organ/tissue are shown in the following table.

TUMOR INCIDENCE

(When > 1/group, or there are other tumors of same organ)

M A L E

# Mice LUNG	64 control	64 5 ppm	64 20 ppm	64 80 ppm	63 200 ppm
Adenoma	2	0	0	0	0
Adenocarcinoma	19	19	19	22	18
LIVER Adenoma	1	3	0	5	2
Hepatic carcinoma	2	4	1	4	2
TOTAL	3	7	1	9	4
SKIN Fibrosarcoma	0	0	0	0	2
Carcinoma of Squamous Cell	0	0	0	0	1
TESTES Seminoma	7	8	3	7	0
Interstitial Cell Tumor	0	0	0	1	0
LEUKEMIA	6	8	9	13	5

F E M A L E

#Mice	64 Control	64 5 ppm	64 20 ppm	64 80 ppm	63 200 ppm
LUNG					
Fibroma	0	0	1	0	0
Sarcoma	0	0	0	1	0
Giant Cell Sarcoma	0	0	1	0	0
Adenocarcinoma	8	5	11	7	10
Metastasis of Hepatic Carcinoma	0	0	0	1	0
LIVER					
Angioma	0	0	0	1	0
Adenoma	0	1	0	0	0
Hepatic Cancer	1	0	0	1	2
Metastasis of pulmonary adenocarcinoma	0	0	1	0	0
SKIN					
Tumor	0	1	0	0	0
Fibrosarcoma	0	0	0	0	3
Adenocarcinoma	1	0	1	2	0
Round Cell Sarcoma	0	1	0	0	0
HIND LEGS					
Sarcoma	0	1	0	0	0
RIGHT THIGH					
Round Cell sarcoma	0	0	0	1	0
UTERUS					
Uterine polyp	1	1	5	2	1
Sarcoma	0	0	1	0	1
Myoma	2	2	1	2	1
Leukemia	27	29	24	24	30

There is no evidence from these data of an oncogenic effect for MITC; however, a final evaluation must await review of these results on the basis of the actual numbers of each tissue examined/sex/group.

Discussion: The possibility of histopathological evidence of a compound effect cannot be definitely evaluated until additional data for the actual numbers of tissues examined are provided. Other than the possibility of a histopathological effect, the only compound effect seen was a slight depressant effect on body weight, and this was minimal. Both sexes of the 200 ppm level showed significant depression of body weight and males of the next lower level (80 ppm) did also. Body weight gains at 26 and 52 weeks for these groups were also significantly less than controls but overall gains (0-106 weeks) were not significantly different for any of the groups. Additionally, the body weight changes for the groups showing the most depression - both sexes of the 200 ppm level - were seldom less than 5-7% lower than their controls.

The biological significance of such a minimal effect can be controversial. Yet it did occur in both sexes of the high level and did show a weak dose-response relationship in that males of the next lower dose level were also affected. Moreover, the slight effect on body weight complies with the concept of a maximum tolerated dose as defined in "Toxicity Potential (Guidance for Anaylsis and Evaluation of Subchronic and Chronic Exposure Studies), EPA - 540/9-85-020, June 1985".

RIN 3426-95

MITC REVIEW

Page____ is not included in this copy.

Pages 13 through 19 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

✓ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.
