Primary Review by Ray Landolt 12/6/94
Review Section I, TB II/HED (7509C) M.L 12/6/94
Secondary Review by Mike Ioannou
Review Section I, TB II/HED (7509C)

DATA EVALUATION REPORT

Study Type: One Year Dog Toxicity Study (83-1)

Test Material: Methyl Isothiocyanate (MITC)

P.C No. 068103
MRID 412407-01

Classification: Wood (Timber) Treatment

Study No. Tox 87246

Date of Study: July 11, 1988

Title of Report: T104 Methyl Isothiocyanate: 1 Year Oral Toxicity Study in Beagle Dogs

Author: R. J. Harling, et al.

Testing Facility: Schering Agrochemicals Limited, England

Sponsor: Nor-Am Chemical Company

Executive Summary: Four groups of six pure-bred beagle dogs/sex/group were dosed orally twice daily with MITC in corn oil at 0, 0.04, 0.4 and 2.0 mg/kg/day for 52 weeks (MRID 412407-01). The NOEL is 0.4 mg/kg/day. The LOEL is 2.0 mg/kg/day with excessive salivation, increased platelet count and activated partial thromboplastin time, decreased blood protein and albumin values and increased liver weight reported at this level.

This study is not acceptable and does not satisfy the Guideline Data Requirement for a nonrodent chronic toxicity study (83-1). It is deficient for the lack of information on the purity of the test material and stability of the use dilutions administered. This study may be upgraded if the requested information is provided and found acceptable. This study is Core Classified - Supplementary.

A. Materials: The test material, supplied in bulk by the sponsor, was formulated as a 1.0% w/v suspension in corn oil in two batches identified as CR 18642/1 and CR 18642/2. The bulk formulations were stored below 30°C in the dark prior to use. Dosing suspensions were prepared twice weekly by the testing laboratory, stored at 4°C then remained at room temperature overnight prior to dosing. Corn oil was used as the suspending agent in preparation of the use dilutions and as the vehicle control. Stability and accuracy of the use dilutions were analysed by the sponsor prior to initiation of the study and for each preparation during the study. Purity of the test material and stability of the use dilutions, to be supplied by the sponsor, were not appended to this report.

Animals: Twenty-four male and 24 female, 22 week old pure-bred beagle dogs weighing approximately 9.6 and 8.4 kg, respectively were used in this study. The animals were acquired from Interfauma UK Ltd, acclimated to the laboratory for 4 weeks during which they were immunized against canine distemper virus, canine adeno virus(hepatitis), para influenza virus, canine parvovirus, Leptospira canicola, Leptospira icterohaemorrhagiae and Bordetella bronchiseptica. Prior to the start of the study and at 3 month intervals the animals were treated with anthelmintic piperazine. All animals were housed individually with tap water available ad libitum. Temperature and humidity were controlled to provide a uniform environment. The dogs were exercised for at least 10 minutes each working day.

B. Study Design: The selection of dose levels for this study were made by the sponsor based on a preliminary study (HRC Report No. FSB 156/85340) not available with this report.

1. Allocation of animals

Group	Dose (mg/kg/day)	Males	Females
1.Control	Corn oil	6	6
2.Low dose	0.04	6	6
3.Mid dose	0.4	6	6
4.High dose	2.0	6	6

The test material was formulated at 0.0004, 0.004 and 0.02g% in corn oil to be administered at a volume of 5 ml/kg twice daily followed by 20 ml of tap water after each dose. There was a 4-5 hour period between daily doses. Due to adverse affects on food intake, body weight and general condition of the animals by week 15 the concentration of the dose levels was increased to 0.008, 0.08 and 0.4g% with a decreased volume of 0.25 ml/kg administered twice daily followed by an equal volume of corn oil. Control animals were treated in a like manner, less the test material.

2. Statistical procedures used for analysis of food intake, bodyweight, organ weight and clinical pathology are attached.

C. Methods and Results

1. Observations:

a. Clinical signs - Individual observations were recorded daily. An increased incidence of vomiting, excessive salivation and liquid feces was observed for the controls and the three test levels during weeks 1-14. These observations were associated with volume of corn oil (5 ml/kg) administered and were particularly severe at the high dose. Following the adjustment to a volume of 0.25 ml/kg during week 15, there was an approximate 4 fold decrease in the incidence of vomiting at the high doses, with a 2% incidence reported in males and females at the high dose for the remainder of the study. Excessive salivation was observed for both sexes at the high dose during weeks 1-15 then increased 3 fold, following the volume adjustment, for the remainder of the study. Liquid feces were observed for both sexes at the high dose during weeks 1-15 decreasing 3 fold with the volume adjustment, remaining at 3% incidence for the duration of the study. MITC is characterized as a severe eye and dermal irritant in the acute toxicity studies. The irritant property of MITC may account for the adverse effects observed.

b. Mortality - No deaths were recorded.

c. Body weight - All animals were weighed weekly during the four week acclimation period, then daily prior to the administration of the first dose. Generally, at the low, mid and high dose levels, males exhibited an increase and females a decrease in body weight gain as compared to the respective control values. A significant ($p<0.05$) decreased body weight gain was reported for females at the high dose during weeks 10-15. The following table summarizes actual (Kg) and percent change in body weight as compared to the controls during weeks 13, 26 and 52.

			mg/kg/day					
	Control		0.04		0.4		2.0	
Week	Male	Female	Male	Female	Male	Female	Male	Female
13	4.4	3.8	6.5 +48	2.7 -29	5.1 +16	2.4 -37	5.1 +16	2.3 -39
26	5.3	4.4	6.4 +21	3.4 -23	4.5 -15	4.0 -9	5.9 +11	3.3 -25
52	4.7	4.5	5.3 +13	3.6 -20	4.3 -9	3.8 -16	5.0 +6	3.0 -33

d. Food consumption - All animals received 400g of standard dry diet each day approximately one hour following the first dose. Anorexia among the control and test groups during the first 15 weeks was associated with the quantity of dose administered. In an effort to stimulate appetite the dry diet was moistened with hot water during weeks 6-9 and/or combined with "tinned meat" during weeks 9-15. During week 15 when the volume of corn oil administered was reduced, appetite was improved and "tinned meat" was discontinued. Moist diet was offered through week 22 followed by a general acceptance of the dry diet through week 52. Dietary intake was adjusted to account for the dry diet equivalent consumed, however, occasionally this was not possible. The number of dogs to receive "tinned meat" in the control, 0.04, 0.4 and 2.0 mg/kg/day levels were 2, 3, 6 and 5, respectively. No dose related correlation in dietary intake was appearent between levels administered.

e. Ophthalmoscopy examinations were performed on each animal initially then during weeks 13, 26 and 52. No treatment related findings were observed.

f. Electrocardiograms were recorded initially on each animal then during weeks 13, 26 an 52. No treatment related findings were recorded for heart rate or electrocardiac function.

2. <u>Clinical Pathology</u> - Blood was withdrawn from the jugular or cephalic vein from all animals initially then during weeks 13, 26 and 52 for determination of hematology and clinical chemistry parameters. All animals were fasted overnight prior to collection of the blood samples.

a. Hematology - The checked (*) parameters are recommended by Subdivision F Guidelines of November 1989.

* Hemoglobin	* Differential count
* Hematocrit	Reticulocyte count
* Erythrocyte count	Mean cell hemoglobin
* Platelet count	Mean cell volume
* Leukocyte count	Mean cell hemoglobin concentration
Cell morphology	Prothrombin time

Activated partial thromboplastin time (APTT)

At the 2.0 mg/kg dose group mean platelet counts for males and females were higher in comparison to the control values during weeks 13, 26 and 52, being statistically significant ($p<0.05$) during weeks 26 for both sexes and during week 52 for males. Group mean APTT values at the 2.0 mg/kg dose were elevated for males as compared to controls during weeks 13, 26 and 52, being statistically significant ($p<0.01$) during weeks 26 and 52. Table 7 from this report is attached.

b. Clinical chemistry - The checked (*) parameters are recommended by Subdivision F Guidelines of November 1989.

* Total Protein
* Urea
* Creatinine
* Cholesterol
* Total bilirubin
* Glucose
* Albumin
* Sodium
* Calcium
* Inorganic phosphorus
* Alkaline phosphatase
  Alanine aminotransferase
* Aspartate aminotransferase
* Lactate dehydrogenase
  Gamma- glutamyltransferase
* Creatinine kinase
  Total glonulin
* Potassium
* Chloride

Total protein and albumin values were significantly ($p<0.05$) reduced for both sexes combined at the 0.4 mg/kg dose during week 52 and at the 2.0 mg/kg dose during weeks 26 and 52. Table 8 from this report is attached.

c. Urinalysis - Urine was collected initially then during weeks 13, 26 and 52. The checked (*) parameters are recommended by Subdivision F Guidelines of November 1989. A random incidence of ketones and oily film on the surface of the urine were reported for animals at the three test levels during week 13, but was not appearent during weeks 26 and 52.

* Volume
* Specific gravity
* Bilirubin
  Total reducing substances
* Glucose
* Blood
* Urobilirubin
* Appearance
* Protein
* Ketones
* Sediment
  pH

3. Terminal findings - On completion of week 52, animals were fasted overnight prior to exsanguination under pentobarbital anesthesia. Dosing was continued over a 4 day period prior to necropsy. The following tissues were collected for histopathological examination and the (x) organs were weighed. The checked (*) parameters were recommended by Subdivision F Guidelines of November 1989.

* Adernals (x)
* GI Tract (esophagus, stomach,duodenum, jejumum,ileum,caecum, colon,rectum)
  Aorta
* Brain (x)(cerebral, cortex,thalamic, medulla,cerebellum)
* Eyes
  Femur & joint
  Gall bladder
* Heart (x)
* Urinary bladder
* Kidneys (x)
  Lachrymal gland
* Liver (x)
* Lungs (x)
* Lymph node
* Mammary gland
* Ovaries (x)
* Pancreas (x)
* Pituitary (x)
  Prostate (x)
* Salivary gland
  Sciatic nerve
* Muscle
  Uterus (x)
* Skin
* Spinal cord (cervical, thoracic, lumbar)
* Spleen (x)
* Testes (x) epididymis,
* Thymus (x)
* Thyroids(x) parathyroid
  Tongue
* Trachea
  Vagina

a. Prior to necropsy, bone marrow smears were obtained from each animal by sternal puncture. An increased incidence of myeloid cells was observed for one dog at the 2.0 mg/kg level.

b. Macroscopic findings were negative for adverse affects.

c. Histopathological examination revealed no pathological findings were observed relative to the dose levels administered.

d. Organ weight - A dose related increase in group mean liver weight was reported for males at the 0.04, 0.4 and 2.0 mg/kg levels, by 2, 8 and 17%, respectively, being significant ($p<0.05$) at the high dose as compared to the control values. A nonsignificant increase in liver weight of 17% was reported for females at the high dose. Table 10 from this report is attached.

D. Discussion Four groups of six pure-bred beagle dogs/sex/group were dosed orally twice daily with MITC in corn oil at 0, 0.04, 0.4 and 2.0 mg/kg/day for 52 weeks. During weeks 1-14 an increased incidence of vomiting, salivation and liquid feces accompanied by reduced body weight gain and anorexia were observed for the controls and three test levels. These adverse effects were associated with the volume of corn oil administered. During week 15 the concentration of the dose was increased and the volume administered was reduced. During the remainder of the study there was a increased incidence of salivation at the high dose with body weight and food intake between the control and test levels comparable. There were no effects on ophthalmoscopy and electrocardiography related to MITC. An increased platelet count and activated partial thromboplastin time accompanied by decreased blood protein and albumin values were reported at the high dose as compared to the controls. A random indicence of ketones and oil film on the surface of the urine were reported for the test levels during week 13, but was not apparent during weeks 26 and 52. No affect on bone marrow, macroscopic or pathological findings were reported. Liver weights were increased for the low, mid and high dose being statistically significant for the high dose males as compared to the controls.

E. Conclusion Four groups of six pure-bred beagle dogs/sex/group were dosed orally twice daily with MITC in corn oil at 0, 0.04, 0.4 and 2.0 mg/kg/day. The NOEL is 0.4 mg/kg/day. The LOEL is 2.0 mg/kg/day with salivation, increased platelet count and APTT, decreased blood protein and albumin values and increased liver weight reported at this level.

This study is CORE Classified - Supplementary and may be upgraded if the purity of the test material and stability of the use dilutions are provided.