SUMMARY OF TOXICOLOGY DATA
Allyl Isothiocyanate

Chemical Code # 1010, Document Processing Number (DPN) 50544
SB 950 # 507
11/25/14

DATA GAP STATUS

Chronic toxicity, rat: No data gap, no adverse effect indicated.
Chronic toxicity, dog: No data gap, not required at this time.
Oncogenicity, rat: No data gap, possible adverse effect.
Oncogenicity, mouse: No data gap, no adverse effect indicated.
Reproduction, rat: Data gap, no study submitted
Developmental toxicity, rat: No data gap, no adverse effect indicated
Developmental toxicity, rabbit: No data gap, no adverse effect indicated
Gene mutation: No data gap, possible adverse effect
Chromosome effects: No data gap, possible adverse effect
DNA damage: No data gap, possible adverse effect
Neurotoxicity: Data gap, no studies submitted

Toxicology one-liners are attached.

All record numbers for the above study types through 279520 (Document No. 50544-0009) were examined. This includes all relevant studies indexed by DPR as of 11/26/14.

In the 1-liners below:
- indicates an acceptable study.
  **Bold face** indicates a possible adverse effect.
  ## indicates a study on file but not yet reviewed.

File name: T141125
Revised by T. Moore, 11/26/14
NOTE: The following symbols may be used in the Table of Contents which follows:
* = data adequately address FIFRA requirement
† = study(ies) flagged as “possible adverse effect”
N/A = study type not currently required

This record contains summaries of studies. Individual worksheets may be useful for detailed assessment.

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METABOLISM AND PHARMACOKINETICS

Metabolism, Rat

50544-0009; 279502; “The Disposition of Allyl Isothiocyanate in the Rat and Mouse”; (Bollard, M., S. Stribbling, S. Mitchell, J. Caldwell; Pharmacology and Toxicology, Imperial College School of Medicine, Norfolk Place, London, UK; Food and Chemical Toxicology 35, 933-943 (1997)); Fischer 344 rats and B6C3F1 mice of both sexes were treated orally by gavage with 2.5 or 25 mg/kg of [14C] allyl isothiocyanate (vehicle: ethanol:Emulphor EL-620:water (1:1:8). Urine, feces and CO2 were collected up to 4 days post-dose. For the 2.5 mg/kg treatment group, 50 to 52% of the administered dose was recovered in the urine of the rats and 79 to 81% for the mice. Recovery in the feces constituted 6 to 12% of the dose in the rats and 7 to 8% in the mice. Radiolabelled CO2 constituted 5 to 6% of the dose for the rats and 3 to 7% of the dose for the mice. For the 25 mg/kg treatment group, 55 to 57% of the administered dose was recovered in the urine of the rats and 77 to 81% for the mice. Recovery in the feces constituted 8 to 10% of the dose in the rats and 6 to 9% in the mice. Radiolabelled CO2 constituted 5 to 6% of the dose for the rats and 7% of the dose for the mice. Sequestration of the radiolabel in the carcass at 4 days post-dose ranged from 19 to 24% for the rats and 2 to 5% for the mice across treatment levels. For the rats, biliary excretion constituted 13 and 8% of the dose for the males and females respectively, up to 6 hours post-dose (treatment level was not reported). Up to 6 hours post-dose, the urinary bladder was the predominant site for the recovery of the radiolabel, approximately 10 fold greater than the concentrations in the liver and kidneys, 20 fold greater than that of the spleen and 100 fold greater than that of the brain. Three major metabolites were identified in the urine, thiocyanate, allyl thio-carbamoylmercapturic acid and allyl thio carbamoylcysteine. The presence of these metabolites varied between the two species with thio carbamoylmercapturic acid being the predominant metabolite for the rat. The thiocyanate moiety constituted the majority of the radiolabel for the mice. A peak concentration of radiolabel in the blood was achieved at 3 hours post-dose for the rat. The excretion was biphasic with an initial half life of 37 hours for the rat and 15 hours for the mouse. The slower phase was characterized by a half life of 140 hours for the rat and 56 hours for the mouse. Summary report. (Moore, 11/19/14)

50544-0009; 279502; “Age-Related Changes in the Metabolism and Excretion of Allyl Isothiocyanate: A Model Compound for Glutathione Conjugation”; (Borghoff, S.J. and L.S. Birnbaum; National Institute of Environmental Health Sciences, Research Triangle Park, NC; Pharmacology and Experimental Therapeutics 14, 417-422 (1986)); Male Fischer 344 rats at 3, 16 and 27 months of age were dosed orally by gavage with 25 mg/kg of [14C] Allyl Isothiocyanate and urine, feces, and CO2 and other volatiles were collected up to 72 hours post-dose. Urine was the primary route for the excretion of the radiolabel with the percentage increasing from 67 to 79% of the administered dose between 3 and 27 months. The remaining radiolabel was distributed between the feces, CO2, and volatile components to a varying degree between the different age cohorts. The radiolabel recovered in the bile up to 6 hours post-dose ranged from 14.5 (27 month old) to 27.5 (3 month old) to 36.7% (16 month old) of the administered dose. These percentages were greater than the percentages of radiolabel which
were recovered in the feces of the uncanullated rats, indicating enterohepatic circulation of the label. The predominant metabolite excreted in the urine was N-acetyl-S-(N-allylthiocarbamoyl)-L-cysteine (68 to 72% of the recovered radiolabel) up to 24 hours post-dose. Five other metabolites were also isolated but not identified. Three of these lesser metabolites were also identified in the bile at 30 minutes post-dose. **Summary report.** (Moore, 11/21/14)
Cancer Epidemiology, Biomarkers & Prevention: 7, 1091-1100 (1998)); Human volunteers consumed horseradish prepared with graded doses of isothiocyanates (predominantly allyl isothiocyanate) at a treatment range of 12.3 to 74 µmol/dose. Approximately 42 ± 5% of the dose was recovered as the dithiocarbamate in the urine within 10 hours of ingestion with a peak level at 1 to 2 hours post-ingestion. Excretion was according to first order kinetics. **Summary report.** (Moore, 11/18/14)

**GUIDELINE ACUTE STUDIES ON ACTIVE INGREDIENT**

**Acute oral toxicity, rat**

50544-0009; 279498; “Acute Oral Toxicity Up and Down Procedure in Rats”; (J. Durando; Product Safety Labs, Dayton, NJ; Study No. 33707; 5/3/12); Female Sprague-Dawley rats were dosed orally by gavage with IR9804 (allyl isothiocyanate) (lot no. C-QJH111109; purity: 99.8%) at doses ranging from 55 to 2000 mg/kg. The following mortality resulted from the treatment: 55 (0/1), 175 (1/2), 550 (1/3), 2000 (2/2). Clinical signs included oral discharge, hypoactivity, prone posture and tremors. Distention of the stomach and discoloration of the intestines were noted in the necropsy examination of those animals which died. LD50 (F): 425.4 mg/kg; Toxicity Category II; **Study acceptable.** (Moore, 11/6/14)

**Acute dermal toxicity**

50544-0009; 279499; “Acute Dermal Toxicity Study in Rats”; (J. Durando; Product Safety Labs, Dayton, NJ; Study No. 33708; 5/2/12); The skin of five Sprague-Dawley rats/sex/group was exposed to 200 or 2000 mg/kg of IR9804 (allyl isothiocyanate) (lot no. C-QJH111109; purity: 99.8%) for 24 hours under an occlusive wrap. Five males and four females in the 2000 mg/kg group died. Clinical signs included hypoactivity, ano-genital staining, and nasal discharge. Dermal irritation and blanching were noted at the application site throughout the 14-day observation period. Discoloration of the intestines and distention of the stomach were noted in the necropsy examination for the animals which died. 200 mg/kg <LD50 (M/F) <2000 mg/kg; Toxicity Category II; **Study acceptable.** (Moore, 11/6/14)

**Acute inhalation toxicity, rat**

50544-0009; 279508; “Acute Inhalation Toxicity Study in Rats”; (C. Lowe; Product Safety Labs, Dayton, NJ; Study No. 33709; 8/30/12); Five Sprague-Dawley derived rats/sex/group were exposed nose-only to 0.206 or 0.508 mg/l (analytical) of IR9804 (allyl isothiocyanate) (lot no. C-QJH111109; purity: 99.8%) for 4 hours. The mean MMAD (GSD) values were 2.11 (2.07), 2.96 (2.51) µm, respectively. One male and one female in the 0.206 mg/l and five males and four females in the 0.508 mg/l exposure groups died. Clinical signs included abnormal respiration, hypoactivity, ano-genital staining, tremors, oral and/or ocular discharge and weight loss. For the animals dying during the observation period, discoloration of the lungs, distention of the stomach and intestines and mottling of the liver were evident in the necropsy examination. No lesions were noted for the animals surviving the observation period. 0.206 mg/l <LC50 (M/F) < 0.508 mg/l; Toxicity Category II; **Study acceptable.** (Moore, 11/10/14)

**Primary eye irritation, rabbit**

Study not submitted.

**Primary dermal irritation**

50544-0009; 279500; “Primary Skin Irritation Study in Rabbits”; (J. Durando; Product Safety Labs, Dayton, NJ; Study No. 33711; 5/2/12); The skin of one New Zealand albino rabbit was exposed to 0.5 ml/site, one site/animal of IR9804 (allyl isothiocyanate) (lot no. C-QJH111109; purity: 99.8%) for 4 hours under a semi-occlusive wrap. Erythema, grade 3 (1/1) was noted at 1
hour post-exposure, increasing to grade 4 (1/1) at 24 hours. Edema, grade 4 (1/1), was noted at 1 and 24 hours post-exposure. The animal was then euthanized for humane reasons. Toxicity Category I. Study acceptable. (Moore, 11/6/14)

Dermal sensitization

50544-0009; 279501; “Local Lymph Node Assay (LLNA) in Mice”; (J. Durando; Product Safety Labs, Dayton, NJ; Study No. 37712; 5/2/12); The dorsal skin on the ears of 5 female CBA/J mice/group was treated by topical application with 25 µl/ear/day of 0 (vehicle: acetone/olive oil (4:1 (v/v)), 2.5, 5.0 or 10% preparations of IR9804 (allyl isothiocyanate) (lot no. C-QJH111109; purity: 99.8%) in the vehicle for 3 days. Additionally, 5 female mice were dosed in the same manner with a 25% preparation of alpha-hexylcinnamaldehyde in acetone/olive oil (4:1 (v/v)) for 3 days. Three days after the last dose, 20 uCi of ³H-thymidine was injected iv into the tail vein of each animal and 5 hours later each animal was euthanized. The draining auricular lymph nodes were removed and single cell suspensions prepared and treated with 5% trichloroacetic acid. The preparations were maintained for 18 hours at 3 to 4°C. Each preparation was then counted using a beta counter and the dpms calculated. A stimulus index (SI) was determined by dividing the mean dpms of each experimental group by the mean value for the vehicle control. An SI value which was greater than 3.0 was considered to a positive response. The SI values for the 2.5, 5.0 and 10.0% preparations were 6.09, 10.31 and 9.11, respectively. The test material is a positive dermal sensitizer. The positive control was functional. Study acceptable. (Moore, 11/7/14)

SUBCHRONIC STUDIES  (units of mg/kg/day unless specified)

Oral toxicity, rat:
Submitted subchronic toxicity studies are included in the review of the chronic toxicity and oncogenicity studies performed by the National Toxicology Program (see below).

Oral toxicity, non-rodent:
Study not submitted nor required at this time.

Dermal toxicity, 21/28-day or 90-day:
Study not submitted nor required at this time.

CHRONIC STUDIES

Chronic, rat

53200 269431, combined study – NTP Technical Report on the Carcinogenesis bioassay of allyl isothiocyanate in F344/N rats and B6C3F1 mice (gavage study); supp; Rat and mouse; National Toxicology Program, Research Triangle Park, NC 27709 and Bethesda, MD 20205, Oct., 1982; food-grade allyl isothiocyanate (greater than 93% purity). Groups of 50 F344/N rats/sex or 50 B6C3F1 mice/sex were given 0 (corn oil as vehicle control), 12 or 25 mg/kg allyl isothiocyanate by oral gavage five times a week for 103 weeks. Rat Single-dose study was done with the test substance on groups of 5 F344/N rats/sex at 25, 50, 100, 200 or 400 mg/kg in corn oil by gavage. All rats survived to the end of the 16-day observation period, and all gained body weights. Toxic signs including inactivity, watery eyes and ruffled fur were observed in the 200 and 400 mg/kg groups males and females. The highest dose for 14-day study was selected at 400 mg/kg due to lack of mortality in the single dose study. Rat Fourteen-day study: Groups of 5 F344/N rats/sex were given the test substance at 25, 50, 100, 200 or 400 mg/kg in corn oil by gavage for 14 days. All rats administered 200 and 400 mg/kg allyl isothiocyanate died during study between days 2 and 9. Body weight gain was reduced in the 100 mg/kg group
males and females at the end of the study. Toxic signs including inactivity and ruffled fur were observed in all dose levels, with the high dose group having the most severe ones. Pathological findings including thickened mucosal surface of stomach and adhesion of the stomach to the peritoneum were observed in groups administered 50 to 400 mg/kg test substance. Therefore, the highest dose for the 13-week study was set at 25 mg/kg. **Rat Thirteen-week study:** Groups of 10 F344/N rats/sex were given the test substance at 0, 1.5, 3, 6, 12 or 25 mg/kg in corn oil by gavage for 13 weeks. No test substance related effects on mortality, histopathology or body weight gains were observed. Doses of 12 and 25 mg/kg were selected for the chronic study in the rats due to pathologic effects in 50 mg/kg group rats in the 14-day study. **Rat Chronic study:** Groups of 50 F344/N rats/sex or 50 B6C3F1 mice/sex were given 0 (corn oil as vehicle control), 12 or 25 mg/kg allyl isothiocyanate by oral gavage five times a week for 103 weeks. Survival at the end of the 103 weeks for male rats: 37/50, 32/50, and 33/50 for the control, 12 and 25 mg/kg dose groups, respectively; for females: 35/50, 29/50 and 33/50 for the control, 12 and 25 mg/kg dose groups, respectively. The mean body weights of the high dose males were lower than those of the controls throughout the study, the mean body weights of the low and high dose females were higher than those of the control group during the last half of the study. Increased rate of urinary bladder transitional cell papilloma was seen in high dose male rats. **Mouse Single-dose study** was done with the test substance on groups of 5 B6C3F1 mice/sex at 50, 100, 200, 400 or 800 mg/kg in corn oil by gavage. Mortality: 2M at 400 mg/kg, 4/5 M, 5/5 F at 800 mg/kg. All surviving mice gained body weights. Toxic signs including inactivity, drooping eyelids and ruffled fur were observed in the 100 mg/kg and above groups males and females. Thickened and necrotic lower third of the mucosal surface of the stomach in male mice at 200, 400 and 800 mg/kg, in female mice at 100, 200 and 400 mg/kg, and adherent stomach to the peritoneal wall in male mice at 400 and 800 mg/kg and in female mice at 200 and 400 mg/kg were observed. The highest dose for 14-day study was selected at 50 mg/kg due to mortality and toxicity in the single dose study. **Mouse Fourteen-day study:** Groups of 5 B6C3F1 mice/sex were given the test substance at 3, 6, 12, 25 or 50 mg/kg by gavage for 14 days. Mortality of 1 male mouse in 50 mg/kg group was observed. Pathological findings including thickened mucosal surface of stomach and thickened urinary bladder wall were observed in groups administered 50 mg/kg test substance. Therefore, the highest dose for the 13-week study was set at 25 mg/kg. **Mouse Thirteen-week study:** Groups of 10 B6C3F1 mice/sex were given the test substance at 0, 1.5, 3, 6, 12 or 25 mg/kg in corn oil by gavage for 13 weeks. No test substance related effects on mortality, histopathology or body weight gains were observed. Doses of 12 and 25 mg/kg were selected for the chronic study in the mice due to pathologic effects in 50 mg/kg group mice in the 14-day study. **Mouse Chronic study:** Groups of 50 B6C3F1 mice/sex were given 0 (corn oil as vehicle control), 12 or 25 mg/kg allyl isothiocyanate by oral gavage five times a week for 103 weeks. Survival at the end of the 103 weeks for male mice: 26/50, 24/50, and 27/50 for the control, 12 and 25 mg/kg dose groups, respectively; for females: 16/50, 25/50 and 18/50 for the control, 12 and 25 mg/kg dose groups, respectively. The mean body weights of the high dose males and females were higher than those of the controls throughout the study. Histopathological examination of liver exhibited dose-related increase in cytoplasmic vacuolation in male mice. Increased rate of lung alveolar/Bronchiolar Carcinoma was seen in high dose male and female mice but was not statistically significant. **Supplemental study.** (Pan &Leung, 5/16/13).

**Chronic, dog**

Study not submitted nor required at this time.

**Oncogenicity, rat**

See Chronic Toxicity, Rat above.
**Oncogenicity, mouse**  
See Chronic Toxicity, Rat above.

**GENOTOXICITY**

**Gene mutation**
53200-0004 269431, Genotoxicity, The Salmonella Mutagenicity test – NTP Technical Report on the Genotoxicity of allyl isothiocyanate, The Salmonella Mutagenicity test, NTP Study ID: 521783; supp; National Toxicology Program, Research Triangle Park, NC 27709 and Bethesda, MD 20205, food-grade allyl isothiocyanate (greater than 93% purity). Strains of Salmonella, TA 98, TA100, TA1535 and TA1537 were tested by preincubation method with or without induced Rat liver S9 (RLI) or Hamster liver S9 (HLI) at concentrations of allyl isothiocyanate up to 1000 ug/plate. Bacterial plus S9 or buffer were incubated for 20 minutes at 37°C with the test material followed with mixing with agar and pouring unto surface of petri dishes containing standard bacterial culture medium. The plates were incubated at 37°C for 2 days and the revertant colonies were counted. The control materials without test substance (dimethyl sulfoxide) and positive control materials were also tested using the same procedure. The results indicated the test substance is negative in inducing reverse mutation with or without metabolic activations (both RLI and HLI) in the strains of *Salmonella* tested. The positive control materials resulted in expected increase in number of revertant colonies and validate the methodology of the test.  
**Supplemental study.** (Pan & Leung, 5/16/13).

53200-0004 269431, Genotoxicity, The Salmonella Mutagenicity test – NTP Technical Report on the Genotoxicity of allyl isothiocyanate, The Salmonella Mutagenicity test, NTP Study ID: 649249; supp; National Toxicology Program, Research Triangle Park, NC 27709 and Bethesda, MD 20205, food-grade allyl isothiocyanate (greater than 93% purity). Strains of Salmonella, TA 98, TA100, TA1535 and TA1537 were tested by preincubation method with or without induced Rat liver S9 (RLI) or Hamster liver S9 (HLI) at concentrations of allyl isothiocyanate up to 400 ug/plate. Bacteria plus S9 or buffer were incubated for 20 minutes at 37°C with the test material followed with mixing with agar and pouring unto surface of petri dishes containing standard bacterial culture medium. The plates were incubated at 37°C for 2 days and the revertant colonies were counted. The control materials without test substance (dimethyl sulfoxide) and positive control materials were also tested using the same procedure. The results indicated the test substance is negative in inducing reverse mutation with or without metabolic activations (both RLI and HLI) in the strains of *Salmonella* tested. The positive control materials resulted in expected increase in number of revertant colonies and validate the methodology of the test.  
**Supplemental study.** (Pan & Leung, 5/16/13).

50544-0009; 279511; “Allyl Isothiocyanate: Mouse Lymphoma”; (National Toxicology Program; Study ID No. 352026; date not provided); Mouse lymphoma L5178Y TK+/- cells were exposed to concentrations of allyl isothiocyanate ranging from 0.05 to 0.8 µg/ml for 4 hours in the first experiment, from 0.2 to 1.0 µg/ml in the second experiment and 0.6 to 1.6 µg/ml in the 3rd experiment under conditions of non-activation. Duplicate cultures/treatment level were included in the study. Cell survival and viability and mutation frequency for each treatment level were determined and compared to those of the solvent control. There was a treatment-related increase in mutation frequency. Positive control was functional. **Possible adverse effect indicated.**  
**Summary report.** (Moore, 11/25/14)

**Chromosome damage**
In vivo assays for detecting Sister Chromatid Exchanges (SCE) were conducted in Chinese hamster ovary cells (CHO-W-B1), in the absence and presence of Acrolor 1254-induced male rat liver S9 enzymes and cofactor mix. CHO cells were incubated with the test chemical at concentrations of up to 0.5 µg/ml for 26 hours without S9 and culture medium. BrdU was added 2 hours after culture initiation. After 26 hours, fresh medium containing BrdU and Colcemid without test material was used to incubate the cells for additional 2 hours. Cells were harvested, fixed and stained at the end of the 2 hour incubation. In the presence of S9 mix, the cells were incubated with the test material, serum free medium and S9 for 2 hours, followed with a 26-hour incubation with medium containing serum and BrdU and no test material. Colcemid was added at the last 2 hours of incubation. Cells were harvested, fixed and stained at the end of the 26 hour incubation. Fifty second division metaphase cells were scored to determine the frequency of SCE/cell for each dose level. Positive control materials Mitomycin C without S9 activation induced expected increases in SCE, while cyclophosphamide with S9 activation induced weak positive response. The test substance was not genotoxic with or without metabolic activation under the conditions tested.

Chromosome aberration (CA) test was done in Chinese hamster ovary cells (CHO-W-B1), in the absence and presence of Acrolor 1254-induced male rat liver S9 enzymes and cofactor mix. CHO cells were incubated with the test chemical at concentrations of up to 5 µg/ml for 8-12 hours without S9 in culture medium. After that, Colcemid was added to culture medium for additional 2 hour incubation. Cells were harvested, fixed and stained at the end of the 2 hour incubation. In the presence of S9 mix, the cells were incubated with the test material and S9 for 2 hours, followed with a 10 hour incubation with fresh medium containing. Colcemid was added at the last 2 hours of incubation. Cells were harvested, fixed and stained at the end of the 10 hour incubation. One hundreds or two hundreds first division metaphase cells were scored to determine the frequency of CA/cell for each dose level. Positive control materials with or without S9 activation induced expected increases in CA. Test material at trial 1 without activation and trial 2 with activation was considered weakly positive for chromosome aberration in the CHO cells. Supplemental study. (Pan & Leung, 5/21/13)

50544-0009; 279512; “In vivo Mouse Bone Marrow Chromosomal Aberrations Test”; (National Toxicology Program; Study ID No. A59578; 11/19/91); Ten male B6C3F1 mice/group were dosed intraperitoneally with 0 (vehicle: corn oil), 25 or 50 mg/kg of allyl isothiocyanate (purity: not reported). A BrdU tablet was implanted subcutaneously at 18 hours post-dose. Two hours prior to euthanasia, colchicine was injected ip. At 36 hours post-dose, the animals were euthanized and the bone marrow in the femurs of 8 animals/group was processed. The marrow cells were scored for aberrations. There was no treatment-related increase in chromosomal aberrations. No adverse effect indicated. The positive control was functional. Summary report. (Moore, 11/14/14)

50544-0009; 279502; “On the Cytotoxicity and Genotoxicity of Allyl and Phenethyl Isothiocyanates and Their Parent Glucosinolates Sinigrin and Gluconasturtiin”; (Musk, S.R.R., T.K. Smith, L.T. Johnson; Institute of Food Research, Norwich Laboratory, Colney, Norwich, UK; Mutation Research 348, 19-23 (1995)); Chinese Hamster Ovary cells (CHO) were exposed to concentrations of allyl isothiocyanate ranging from 2.4 to 3.0 µg/ml for 1 hour, washed and incubated for an additional 16 hours for the chromosomal aberration assessment and for 32 hours in the presence of 10 µM BUdR. There was no treatment-related increase in chromosomal aberrations and no increase in sister chromatid exchanges. No positive control was included in the assay. The exposure to the test material was less than that recommended in the OPPTS guideline, 870.5375 of 3 to 6 hours. No metabolic S9 fraction was included in the
assay. No extended exposure period of 18 to 24 hours (1.5 cell cycles) was included in the assay after the negative response noted in the shorter exposure for the non-activated assay. These data are not adequate to assess the potential genotoxicity of the test material. **Summary report.** (Moore, 11/20/14)

**DNA damage or miscellaneous effects**

53200-0004 269431, Genotoxicity, NTP Technical Report on the Genotoxicity of allyl isothiocyanate, Bone marrow Micronucleus, NTP Study ID: 896173; supp; B6C3F1 mouse, National Toxicology Program, Research Triangle Park, NC 27709 and Bethesda, MD 20205, food-grade allyl isothiocyanate (greater than 93% purity). Groups of 5 male B6C3F1 mice were administered with test substance at 37.5, 75 or 150 mg/kg by intraperitoneal injection for three times over 72 hours of period. Blood samples were obtained from each dosing group 24 hours after the injection and slides were prepared, fixed and stained for micronucleus. The test substance was not genotoxic under the conditions tested. Vehicle control (corn oil) and positive control material (Dimethylbenzanthracene at 12.5 mg/kg) were administered to 5 male mice/group in the same manner. Two thousand polychromatic erythrocytes (PCEs or immature erythrocytes) were counted and the numbers of micronucleated PCEs were indicators of possible genotoxicity in inducing micronucleus in peripheral blood. Positive control material induced a higher micronucleated PCE compared to vehicle control, there was no test material treatment related increase in micronucleated PCEs. **Supplemental study.** (Pan & Leung, 5/21/13).

50544-0009; 279505; “Lack of UDS Activity in the Livers of Rats Exposed to Allylisothiocyanate”; (Bechtel, D., L. Henderson, R. Proudlock; CanTox U.S. Inc, Bridgewater, NJ, Unilever Research, SEAC Toxicology, Sharnbrook, Bedford, England, Huntington Life Sciences, Ltd., Huntingdon, Cambridgeshire, England; *Teratogenesis, Carcinogenesis, and Mutagenesis* 18, 209-217 (1998)); Male Hsd/Ola Sprague Dawley rats were dosed orally by gavage with 37.5 and 125 mg/kg of allyl isothiocyanate (vehicle: corn oil). Animals were euthanized at 2 and 14 hours post-dose. Hepatocytes were harvested and cultures were prepared. Cells were incubated with (methyl-³H)thymidine for 4 hours, followed by an additional 24 hour incubation period. Cells which were attached to coverslips were fixed and mounted onto slides. The presence of unscheduled DNA synthesis was examined by autoradiography. Net nuclear grain count was determined by enumerating the number of grains in the nucleus as compared the number in the cytoplasm. There was no increase in the net nuclear grain count indicative of unscheduled DNA synthesis. The two positive controls were functional. **Summary report.** (Moore, 11/20/14)

50544-0009; 279505; “Neoplastic Transformation of Chinese Hamster Cell in vitro after Treatment with Flavoring Agents”; (Kamasaki, A., T. Yasuhara, S. Urasawa; Department of Hygiene, Sapporo Medical College, Chuo-ku, Sapporo, Japan; *Journal of Toxicological Sciences* 12, 383-396 (1987)); Chinese Hamster B241 cells and HAIN-55 human fibroblasts were exposed to 5 nM and 20 nM concentrations of allyl isothiocyanate (AITC), respectively. The cells were cultured for succeeding generations until they had acquired the characteristics of transformed cells (i.e., increase in saturation density in monolayer culture, plating efficiency at a low serum level, and colony forming efficiency in soft agar medium (anchorage independence). Anchorage independent cells were isolated and cultured for injection into nude mice (BALB/c, JCL, NuNu). The mice were inoculated with 1x10⁵ cells by means of a subcapular subcutaneous injection. When the resulting tumors were approximately 2 cm in diameter, they were excised and examined histologically. In addition, a single cell suspension of the tumor cells were prepared and injected into a new host. The time to tumor formation was determined. In the case of the HAIN cells, exposure and repeated culturing did not result in transformed...
cells. The CH B241 cells were transformed. There was an increased frequency of chromosomal aberrations and the transformed cells were tumorigenic when injected into new hosts. **Possible adverse effect:** CH B241 cells were transformed and demonstrated tumorigenicity; however, the HAIN-55 cells were resistant to transformation. **Summary report.** (Moore, 11/21/14)

50544-0009; 279503; “Detection of Chemical Mutagens by the Dominant Lethal Assay in the Mouse”; (S.S. Epstein, E. Arnold, J. Andrea, W. Bass and Y. Bishop; Laboratories of Environmental Toxicology and Carcinogenesis and Laboratories of Biostatistics, Children’s Cancer Research Foundation, Inc., and Department of Pathology, Harvard Medical School and Department of Biostatistics, Harvard School of Public Health, Boston, MA; Toxicology and Applied Pharmacology, 23, 288-325 (1972)); The report documented the testing of Allyl Isothiocyanate among other chemicals for potential mutagenic effects upon male gametes in the dominant lethal assay. Seven and nine male ICR/Ha Swiss mice were dosed by intraperitoneal injection with 3.8 and 19 mg/kg of allyl isothiocyanate (no purity was provided), respectively. Two and 7 animals died during the 8 week mating period. The test material was classified as producing early fetal deaths and preimplantation losses within control limits. However, there was an excessive number of animals which died during the study and no specific study data were provided to document the treatment results (i.e., effect on implantation and/or fetal survival). The data are not sufficient to adequately assess the potential for the test material to induce dominant lethal mutations. **Summary report.** (Moore, 11/10/14)

**REPRODUCTIVE TOXICITY, RAT**
Study not submitted.

**DEVELOPMENTAL TOXICITY**

**Rat**

53200-0006 269433, teratology study – Teratologic evaluation of FDA 71-26 in Mice, Rats, Hamsters and Rabbits, Oil of Mustard; supp; Mice, Rats, Hamsters and Rabbits, Food and Drug Research Laboratories, Inc., Maspeth, NY 11378, 4/16/1973; FDA 71-26 (Oil of mustard), a clear dark brown liquid, was administered once daily by gavage to mated adult albino CD-1 outbred female mice from day 6 to day 15 of gestation (or to mated adult albino Wistar derived female rats from day 6 to day 15 of gestation, or to mated golden hamster from day 6 to 10 of gestation, or to artificially inseminated Dutch-belted female rabbits from day 6 to day 18 of gestation). Doses (in corn oil) given to pregnant mice were: 0, 0.3, 1.3, 6.0 and 28.0 mg/kg, positive control material Aspirin: 150 mg/kg; for pregnant rats were: 0, 0.2, 0.85, 4.0 and 18.5 mg/kg, positive control material Aspirin: 250 mg/kg; for pregnant hamsters: 0, 0.2, 1.1, 5.1 and 23.8 mg/kg, positive control material Aspirin: 250 mg/kg; for pregnant rabbits: 0, 0.123, 0.6, 2.8 and 12.3 mg/kg, positive control material 6-amino nicotinamide: 2.5 mg/kg. On day 17 (or day 20 for rats, day 14 for hamster and day 29 for rabbits) all dams were subjected to Caesarean section under anesthesia, and the numbers of implantation sites, resorption sites, and live and dead fetuses were recorded. The fetal weights, visceral and skeletal defects were recorded or examined. Increased total resorption, and numbers of dead fetuses were observed in the 28 mg/kg dose group mice offspring. Increased litter and fetal incidence of incomplete ossification of sternebrae in 23.8 mg/kg FDA 71-26 treated Hamsters. No effects at HDT were observed in treated rabbits and rats. NOEL (No Observed Effect Level): Rabbit [Maternal and Developmental NOEL: 12.3 mg/kg based on no effects at HDT]; Hamster [Maternal NOEL: 23.8 mg/kg based on no effect at HDT; Developmental NOEL: 5.1 mg/kg (based on incomplete ossification of sternebrae)]; Rat [Maternal and Developmental NOEL: 18.5 mg/kg based on no
effects at HDT]; Mice [Maternal NOEL: 6.0 mg/kg based on increased litters with resorption sites; Developmental NOEL: 6.0 mg/kg based on increased fetal deaths]. Supplemental study. (Pan & Leung, 5/24/13).

50544-0009; 279515; “Correlation of Teratogenicity and Molecular Structure: Ethylenethiourea and Related Compounds”; (Ruddick, J.A., W.H. Newsome, L. Nash; Food Protectorate, Health Protection Branch, Ottawa, Canada; Teratology 13, 263-266 (1976)); Five mated female Wistar rats were dosed orally by gavage with 60 mg/kg of allyl isothiocyanate (AITC) on day 12 or 13 of gestation. Sixty mg/kg was selected because higher doses killed some of the dams. However, in the Results and Discussion Section of the paper, the author stated that the dose was doubled, possibly because no effects were evident at the stated dose. It was unclear as to whether that meant the animals might have been dosed with 120 mg/kg. No treatment-related effects were noted for the test material. The study is inadequate for assessing the teratogenic potential of AITC as only a single dose was administered. Summary report. (Moore, 11/24/14)

Rabbit
See Rat Developmental Toxicity above.

NEUROTOXICITY

Acute neurotoxicity, rat
Study not submitted.

90-day neurotoxicity, rat
Study not submitted.

Developmental neurotoxicity, rat
Study not submitted.

Delayed neurotoxicity, hen
Study not required.

IMMUNOTOXICITY
Study not required at this time.

ENDOCRINE DISRUPTOR STUDIES
Studies have not been submitted.

SUPPLEMENTAL STUDIES
50544-0009; 279496; “Allyl Isothiocyanate-Rich Mustard Seed Powder Inhibits Bladder Cancer Growth and Muscle Invasion”; (A. Bhattacharya, Y. Li, K.L. Wade, J.D. Paonessa, J.W. Fahey, Y. Zhang; Department of Cancer Prevention and Control and Department of Urology, Roswell Park Cancer Institute, Buffalo, NY, Department of Pharmacology and Molecular Sciences and Center for Human Nutrition, The Johns Hopkins University, Baltimore, MD; Carcinogenesis 31 no. 12, pp. 2105 – 2110 (2010)); Allyl isothiocyanate (AITC) is present in Mustard Seed Powder (MSP-1) as its glucosinolate precursor, sinigrin. It was assessed as to its potential to inhibit bladder cancer development in Fischer 344 female rats. The bladders of these animals were inoculated with AY-27 rat bladder cancer cells. In this model, the AY-27 cells have been shown to aggressively establish themselves orthotopically. Beginning one day later and for the succeeding 3 weeks, three groups of animals were dosed orally with the
vehicle, 71.5 or 715 mg/kg/day of MSP-1 (9 and 90 µmol/kg of sinigrin, respectively). Within this 3-week period, the bladder weight of the control group was 5 times that of a rat which had not been inoculated with the cancer cells, demonstrating the tumorigenic capacity of the AY-27 cells. The cancer cells had also invaded the musculature of the bladder in most cases. Treatment with 71.5 and 715 mg/kg of MSP-1 resulted in mean bladder weights which were 35 and 23% less than that of the control. In the histopathological examination, no invasion of the musculature was noted for the 71.5 mg/kg treatment group. For the 715 mg/kg group, cellular invasion of the musculature was noted at practically the same incidence level as that for the control group. Treatment with 71.5 mg/kg of MSP-1 resulted in a reduction in the vascular endothelial growth factor (VEGF) and increased activation of Caspase 3 and cleavage of poly ADP ribose polymerase (PARP). These observations were deemed to be apropos for any therapeutic anticancer potential which AITC may have. A more optimal treatment level was at 71.5 mg/kg of MSP-1 rather than at 715 mg/kg of the test material. **Summary Report.** (Moore, 11/12/14)