APPENDIX D

TOXICOLOGY SUMMARY FOR METHYL BROMIDE

CALIFORNIA DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA

NAME OF ACTIVE INGREDIENT:
METHYL BROMIDE

Chemical Code # 385, SB 950 # 078, Tolerance # 123

October 20, 1987
Revised May 10, 1989; July 12, 1991; Jan. 17, 1992; Feb. 5, 1993;
July 24, 1995; October 29, 1997; and March 5, 1999

I. DATA GAP STATUS

Chronic rat: [1]	No data gap, possible adverse effect
Chronic dog:	No data gap, possible adverse effect [2]
Onco rat: [1]	No data gap, possible adverse effect [3]
Onco mouse:	No data gap, possible adverse effect [3]
Repro rat:	No data gap, possible adverse effect
Terato rat:	No data gap, possible adverse effect
Terato rabbit:	No data gap, possible adverse effect
Gene mutation:	No data gap, possible adverse effect
Chromosome:	No data gap, possible adverse effect
DNA damage:	No data gap, possible adverse effect
Neurotox:	Inadequate study, possible adverse effect indicated [4]

[1] Record 158746 was a chronic toxicity-oncogenicity study using feed containing microencapsulated methyl bromide.
[2] See Note under "Chronic, Dog" dated 3/5/99 by Gee
[3] A summary publication of 2-y bioassays done by inhalation with F344 rats and BDF1 mice (record 143994) suggests that methyl bromide increased some tumor incidences; other bioassays using Wistar rats (record 059184) and B6C3F1 mice (record 116243) did not indicate an oncogenic effect for inhalation exposure.
[4] Under SB950, this category is for acute delayed neuropathy testing in hens for agents with anticholinesterase activity. Since this does not apply to methyl bromide, there is no data requirement. The study in question here refers to an 827-type, 90-d rat study that USEPA called in.

Note: Toxicology one-liners are attached.
** indicates acceptable study.
Bold face indicates possible adverse effect.

Revised file name: T990305
Revised by: Stephen J. Rinkus and Joyce Gee (3/5/99)

EPA Reregistration guidance document dated August, 1986 contains EPA findings.

Stephen J. Rinkus 3/5/99

Joyce Gee 3/5/99

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

CHRONIC, RAT

****123-179 158746** "A 24-Month Chronic Dietary Study of Methyl Bromide in Rats" (Dr. Jozef J.W.M. Mertens; WIL Research Laboratories, Inc.; laboratory study number: WIL-49014; 12/9/97). Corn oil containing methyl bromide was microencapsulated using starch and sucrose. Two types of microcapsules were produced. One was a blend of 7 production runs; it had a methyl bromide content of 0.48% w/w. The second type was a blend of five production runs; its methyl bromide content was 3.44% w/w. The two types of microcapsules differed also in terms of corn oil, starch, and sucrose contents and age of the material at start of testing. The microcapsules were dispersed into granular feed for presentation to the animals. Nominal methyl bromide concentrations in the diet were as follows: 0 (basal diet), 0 (diet containing placebo microcapsules), 0.5 , 2.5, 50 and 250 ppm. The blend containing 0.48% methyl bromide was used to prepare the two low doses while the blend containing 3.44% was used to prepare the two high doses. The highest dose tested was selected on the basis of a two-week range-finding study that also is on file (record 162360). The daily ration of feed varied as follows: for test weeks 0-65, males and females each received 30 and 23 g, respectively; for test weeks 66 -104, males and females received 35 and 30 g, respectively. One outcome of this feeding strategy appears to have been that a fraction of the animals in the control and 0.5 to 50 ppm groups had their feed consumption restricted during the first 65 weeks of the study. The number of rats/sex/dose level was 50-70 at the start of the study. In test week 53, interim sacrifices were performed on 18-20 rats/sex for the following dose levels: 0 (basal diet), 0 (placebo microcapsules), 50 and 250 ppm. Survival was statistically increased in the 250 ppm male group and in the 50 and 250 ppm female groups when compared to the placebo-microcapsule groups. Bodyweight was reduced in the 250 ppm groups; the reduction reached a maximum in the first weeks of testing in both sexes; a further reduction in bodyweight relative to the controls (placebo microcapsule groups) did not occur despite continued exposure. Since a reduction in feed consumption occurred in the 250 ppm groups (both sexes) starting with the first exposure week, the bodyweight reduction would appear to be due mainly to the reduced feed consumption. No treatment-related effects were reported in the following areas: clinical observations, ophthalmology, hematology, serum chemistry or urinalysis. Effects on absolute organ weights (brain, kidneys, liver, testes/ovaries) and organ weights relative to bodyweight appeared to be due to the bodyweight reduction in the 250 ppm groups; this was true for animals sacrificed at test week 52 as well as for the survivors at the end of the study. Possible, treatment-related findings at necropsy were an increasing incidence of splenomegaly in the males (0 ppm, basal: 2/50; 0 ppm, placebo: 2/50; 0.5 ppm: 7/50; 2.5 ppm: 10/50; 50 ppm: 11/50; and 250 ppm, 3/50) and an increased incidence of dark red areas on the liver in the 50 ppm females surviving to test week 104 (0 ppm, basal: 5/20; 0 ppm, placebo: 3/19; 0.5 ppm: 8/22; 2.5 ppm: 4/24; 50 ppm: 14/27; and 250 ppm, 8/29). No statistical analyses were supplied for the histology data. Also, the lesion-incidence summary table did not present autolysis and lesion-grade data and may not have been corrected for tissues lost to autolysis. Possible treatment-related effects include: increased incidence of pancreatic acinar atrophy at 250 ppm (both sexes), increased incidence of adrenal cortical hypertrophy at 250 ppm (females), and increased incidence of pulmonary arterial mineralization at 50 ppm (females). Two rare tumor types, adenocarcinoma of the prostate and endometrial stromal sarcoma of the cervix, were seen at 4% incidence at 250 ppm. By experimental design, the histological examinations of the pancreas, prostate, spleen, adrenal glands, cervix, and uterus at the 0.5 to 50 ppm dose levels were limited to those rats that did not survive to terminal sacrifice. Autolysis was a frequent observation in the GI-tract organs in rats that did not survive to the end of the study (all groups, both sexes). While an increased incidence of spongiosis hepatis was seen in the 50 ppm females, the relationship of this lesion to angiectasis and the necropsy finding of dark red liver spots that also occurred at the 50 ppm dose level needs clarification. When first reviewed (Rinkus, 3/20/98), the study was considered unacceptable pending the submission of the supplemental information described in worksheet W158746.835 regarding: range-finding study; analytical methods; cause and extent of autolysis; histological examinations

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for the lower dose groups; and clarification of liver gross and histological findings. Subsequently, records 160305, 162360, 162361 and 165140 were submitted. For the reasons discussed in worksheet W158746.S03, this study is now considered marginally **ACCEPTABLE, with a LOEL = 0.5 ppm.** This is a conservative call based on the following: in the absence of histological data to the contrary, the instances of splenomegaly in the 0.5, 2.5 and 50 ppm male groups that have not been examined histologically are assumed to be due to lymphoma. (Rinkus, 3/5/99)

123-182 160305 This record was the response from the Registrant to the February 20, 1998 review of record 158746. It was received at DPR on March 30, 1998. The initial section consisted of 11 pages of narrative, two small tables, and one large table showing group bodyweight gains as a percentage of feed consumed on weekly basis from test week -2 to test week 104 (both sexes). The initial section addressed the following issues related to the first DPR MT review: selection of 250 ppm as the high dose; why decreased bodyweight in the 250 ppm groups would not be explained by a decrease in feed consumption due to an olfactory-aversion mechanism; the bioavailability of the methyl bromide in the microcapsules; clarification of analytical methods; and a defense of the practice of not examining all gross lesions in the study. The final section of record 160305 concerned the analytical method. It further explained the methods, addressed specific issues raised in the first DPR MT review and provided five "exhibits," which were sets of raw data and chromatograms in support of positions taken in the narrative portion. This record is discussed in worksheet W158746.S03. **Supplementary information.** (Rinkus, 3/5/99).

123-187 162361 "Determination of the Stability of Microencapsulated Methyl Bromide in Diet" (Severs, L. W.; laboratory study number: WIL-49010; May 9, 1994). This is an analytical study that was referenced in record 160305. This study was supposed to be the basis for the strategy in record 158746 of heating at 100°C for 15 minutes preinjection when assaying feed containing microencapsulated methyl bromide. This record is a short report in the form of a letter (6 pages of narrative, 2 tables) from Loren W. Severs (Manager of Analytical Chemistry, WIL Research Laboratories) to Kathryn Rosica (Methyl Bromide Industry Panel, Chemical Manufacturers Association). It is notable for the following: 1) it provides no data or discussion per se supporting the selection of 100°C for 15 minutes as the preinjection heating procedure; 2) the stability analysis indicated that after 24 h at room temperature, the methyl bromide content of feed containing microencapsulated methyl bromide (100 ppm) was 80% of the content after preparation whereas no loss of methyl bromide occurred in this interval when the feed was stored in the freezer; and 3) $\geq$ 10 minutes of heating feed containing microencapsulated methyl bromide at 54°C results in the formation of methyl chloride. This record is discussed in worksheet W158746.S01. **Supplemental information.** (Rinkus, 11/17/98).

123-186 162360 "A Two Week Dietary Range-Finding Toxicity Study of Methyl Bromide in Rats " (Mertens, J.J.W.M.; laboratory study number: WIL-49015; April 9, 1996). This study was the basis for the selection of the high dose in record 158746. For 18 days, 5 Crl:CD®BR rats/sex/dose were fed basal diet or a diet containing 250 ppm methyl bromide presented as microcapsules dispersed in feed. The microencapsulated material was ill defined; apparently, it was obtained from Pharmaco LSR, Inc. and had a methyl bromide content of 6.1%. All rats survived to scheduled sacrifice and were necropsied. The only treatment-related effects were decreased bodyweight and decreased food consumption in the 250 ppm male group. The relevance of this study in terms of the selection of 250 ppm as the high dose in record 158746 is questionable due to the following considerations: the duration of exposure was only 18 days; it is not clear if the methyl bromide content of the microcapsules was determined; and it was not addressed whether the microencapsulated material used in this study was comparable to the microcapsules used in record 158746. This record is also notable because the analytical strategy was similar to that used in record 158746 and involved headspace analysis with heating for 15 minutes at 100°C preinjection. It was indicated that as part of the quantitation of methyl bromide, the conversion to methyl chloride was taken into account. **Supplemental information.** This record is discussed in worksheet W158746.S02. (Rinkus, 11/20/98).

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123-207 165140 This record was the response from the Registrant to the March 20, 1998 review of record 158746. The initial section was a 6-page narrative addressing: the bioavailabilty of methyl bromide when using microencapsulated material; and specific items discussed in memorandum M980512, dated May 12, 1998, from the DPR MT reviewer (Dr. Rinkus) to Gary Patterson (Medical Toxicology Branch Chief) regarding the analytical methods used in record 158746. Following the initial section were four attachments concerning:1) literature citations for other toxicological studies wherein an agent was tested using microencapsulation; 2) a discussion of the pathology data as a justification for not conducting the histological examinations requested in the March 20, 1998 review of record 158746; 3) data from Midwest Research Institute for the February, 1994 titering of the 0.48% microcapsules; and 4) data from Midwest Research Institute for the January, 1995 titering of the 3.44% microcapsules. This record is discussed in worksheet W158746.S03. **Supplementary information.** (Rinkus, 3/5/99).

123-127 095929 "Two-Year Oral Chronic Toxicity and Carcinogenicity Study in Rats of Diets Fumigated with Methyl Bromide," (Mitsumori et al., Fd. Chem. Toxic. 28:109-119, 1990). This study used F344 rats (both sexes) to examine the chronic toxicity and carcinogenicity of methylation products and bromine residues resulting from fumigation of rat feed with methyl bromide. After fumigating the feed to attain ~500 ppm total bromine, the feed was exposed to air for 3 weeks; this feed was then pulverized and mixed with untreated feed to achieve dose levels of total bromine of 200 and 80 ppm. Actual organic methyl bromide levels were not determined in this study, except to note that at the end of the 3-weeks airing, the level of organic methyl bromide in the feed containing ~500 ppm total bromine was < 20 ppm. The only effect observed in this study was body weight depression in males fed the diet containing 500 ppm total bromine; the effect was attributed to methylation products generated in the feed since a comparable effect was not seen in rats fed a diet containing 500 ppm KBr. **Supplemetary information. No workheet.** (Rinkus, 5/3/91).

123-157 131601 "Draft Protocol: A 24-Month Oral Chronic Toxicity Study of Methyl Bromide in Rats" (no author identified; WIL Research Laboratories, Inc.; no study/project/report number; April 27, 1993). This record is an unsigned "draft" protocol for a chronic toxicity study in Crl:CD(SD)BR rats (both sexes). The proposed route of administration is by gavage using corn oil solutions through which methyl bromide has been bubbled. Not reviewed (unsigned draft proposal). **Supplementary information. No workheet.** (Rinkus, 7/24/95).

SUBCHRONIC, RAT

123-043 913094 A 90-day subchronic rat study (Danse et al., Tox. Appl. Pharm. 72: 262-271, 1984) indicated a carcinogenic response in forestomach at 50 mg/kg. (Wong, 4-8-85). However, a reanalysis of the histological slides of Danse et al. by a NTP panel concluded that the lesions appeared to be nonneoplastic only (inflammation and hyperplasia) (see letter of 5/9/84 from Dr. Boorman [NTP] to Dr. Vos [National Institute of Public Health, The Netherlands] in front of CDFA document 123-103). (Rinkus, 4/25/89). However, while Hubbs (record 059183 in CDFA document 123-083) also did not find any carcinogenicity in rats treated up to 17 weeks with 50 mg/kg, Boorman et al. (Toxicol. Applied Pharmacol. 86: 131-139, 1986) did observe an early carcinoma in one of 11 rats treated for 25 weeks at 50 mg/kg. (Rinkus, 4/17/90).

NOTE The memo from EPA to CDFA addressing differences in data gap status for this chemical (dated 2/17/89) notes EPA classification as "Core Minimum". CDFA reviewer (Aldous) presumes this to refer only to the subchronic study data requirement, since the 1986 Registration Standard did not consider the chronic rodent study data gap filled. [Aldous, 1/5/90].

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123-109 087805 "Histopathology of Acute Toxic Responses in Selected Tissues from Rats Exposed by Inhalation to Methyl Bromide," (Hurtt et al., Fund. Applied Toxic. 9:352-365, 1987). Methyl bromide (99.9% pure) was given by inhalation to groups of 10 adult male Fischer 344 rats at 0 (air), 90, 175, 250, and 325 ppm for 6 h/day for 5 days; an additional untreated group received feed quantities identical to those consumed by the rats in the 325 ppm group. After the 5th exposure or in extremis (325 ppm, 4 days), rats were sacrificed and the following sites were examined histologically: nasal cavity, brain, liver, kidney, adrenal glands, testes, and epididymides. Ataxia and diarrhea were observed in the rats exposed to ≥ 250 ppm; tremors and/or convulsions were observed in a few rats exposed to 325 ppm; reddish perineal staining (hemaglobinuria ?) in some rats exposed to ≥ 175 ppm; no clinical effects were cited for rats exposed to 90 ppm. Histological findings were: degeneration of the nasal olfactory epithelium (≥ 175 ppm); degeneration in the cerebellar cortex (≥ 175 ppm; two lesions noted: large to small foci of granule cells, with edematous distension of the cytoplasma; and a diffuse granule cell degeneration without the edematous cytoplasma); degeneration in the cerebral cortex (325 ppm) and the dorsolateral regions of the thalamus (325 ppm); hepatocellular degeneration (325 ppm); lipid accumulation in parenchymal cells of adrenal cortex (≥ 175 ppm); and delayed spermiation (325 ppm). No lesions were noted in the kidneys or the epididymides; the former finding indicates that the presumed hemoglobinuria is not due to a renal lesion. The authors compared these lesions to similar lesions seen in rats exposed to methyl chloride (which presumably was at much greater concentrations, e.g., 3000 ppm). Supplemental information. No worksheet. (Rinkus, 2/28/90).

ACUTE, RAT

123-162 132699 "Acute Oral Toxicity Comparison of Microencapsulated Methyl Bromide and Liquid Methyl Bromide in Albino Rats" (Kiplinger, G.R.; laboratory study number: WIL-49011; September 22, 1994). Two types of corn oil solutions were compared: one made with methyl bromide (added as a liquid) and the other made with microencapsulated methyl bromide. In the liquid methyl bromide testing, methyl bromide, 99.5% purity, was given once by gavage to 5 Crl:CD®BR rats per sex per dose level at 50, 100 and 150 mg/kg in initial testing and at 0, 80, 120 and 160 mg/kg in retesting. The initial dose levels were chosen on the basis of range-finding testing which also was discussed in the report. Rats were fasted 18-20 hours prior to dosing and feed was made available 3-4 hours after dosing. Rats were observed for mortality and clinical signs at approximately 1, 3 and 4 h after dosing (postdosing day 0) and once in the morning and once in the afternoon on postdosing days 1 through 14 (day of scheduled sacrifice). All rats in the initial testing and retesting were necropsied. In the retesting, microscopic examination of the stomach, duodenum, jejunum and ileum also was performed. With one exception, rats that died did so on or before postdosing day 2. At necropsy, the main organ affected was the stomach. The findings were consistent with severe irritation of the lumen surface. The mortality data indicated a slight sex difference; for example, LD50 values (method of Litchfield and Wilcoxon) for males and females in the initial testing were, respectively, 139 mg/kg (125-155 mg/kg as 95% confidence interval) and 107 mg/kg (97-119 as 95% confidence interval). The lowest LD50 value was 86 mg/kg (77-96 mg/kg as 95% confidence interval); this was seen in the females in the retesting. The testing of microencapsulated methyl bromide can not be evaluated pending clarification of the following: 1) whether the microcapsules dissolved before dosing; 2) whether the procedure for the methyl bromide content analyses was appropriate; and 3) whether the microencapsulated material was comparable to the material used in the two-year microencapsulated methyl bromide feed study, record 158746. **Supplemental information.** (Rinkus, 11/13/98).

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CHRONIC, DOG

Note: As has been done with other active ingredients, the collective data for toxicity studies with a non-rodent were evaluated. Although no single study has been found acceptable, no further studies are being required at this time and the data gap is considered filled, with possible adverse effects noted in several studies, as indicated in the following summaries of the individual studies (Gee, 3/5/99).

Note: Chronic-toxicity testing using inhalation as the route of exposure is no longer being required (see rebuttal response of July 24, 1995). (Rinkus, 10/29/97).

123-175 143945 "A Chronic (12-Month) Toxicity Study of Methyl Bromide Fumigated Feed in the Dog" (Newton, P.E.; Pharmaco LSR.; study no. 94-3186; 1/4/96). Granular feed containing 10% corn oil was fumigated with methyl bromide at concentrations of 0, 7092, 20,000 or 116,279 ppm for one hour. After one hour of degassing, the feeds were presented to four beagle dogs/sex/dose level (except 8 dogs/sex at the high dose). Fumigated feeds were presented five d/week for one year. Nominal residual methyl bromide levels in the feed-corn oil admixture one hour after the feed had been presented to the dogs were: 0, 0.5, 1.5 and 5.0 ppm. In addition, test feeds presumably contained fumigation-derived products (bromide, methylation adducts, methyl chloride). While the concentrations of reaction products were not measured, because of the experimental design, their concentrations in the low-dose feed versus high-dose feed may have varied by a factor of 16. Residual methyl bromide levels were selected on the basis of discussions between the Registrant and the USEPA to achieve a "safety" study (i.e., the high dose was not set on the basis of toxicity data). A new analytical procedure was developed to determine residual methyl bromide; however, the adequacy of the new procedure could not be assessed pending submission of supplemental information. There were no clear effects on survival, cageside observations, bodyweight or food consumption. Possible treatment-related effects included: decreased hemoglobin and (or) hematocrit at 3, 6 and (or) 12 months in the high-dose male group; and decreased serum calcium at 6 and 12 months in the mid- and high-dose male group. The incidence of thyroid C-cell hyperplasia in the male control group was 1/4 versus 5/8 in high-dose male group; histological examination of thyroids from mid- and low-dose males was not done. Statistically reduced absolute kidney weight was seen in the mid- and high-dose female groups; when viewed relative to terminal bodyweight or brain weight, kidney effects were not statistically significant. Due to the experimental design, the effects seen in this study may be due to residual methyl bromide and (or) its reaction products. **NOEL = 1.5 ppm (anemia).** When first reviewed (Rinkus, 9/5/97), this study was considered unacceptable and upgrading would require the submission of the following: 1) supplemental information regarding the analytical method; 2) historical control data for thyroid C-cell hyperplasia in males; 3) histological examination of the thyroid in the low- and mid-dose male groups and the parathyroid in three high-dose females whose tissues were not examined originally; and 4) the statistical analyses of the hemoglobin, hematocrit and serum phosphate data. Subsequently, the Registrant submitted records 165489 and 165490 (dated 12/23/98 and 12/29/98). The former contains data regarding the analytical method; the latter contains histological data for the thyroid and parathyroid and historical-control data for the thyroid. Based on the newly submitted data, C-cell hyperplasia has been dropped as a possible adverse effect. Validation for the analytical method is requested (discussed in worksheet W143945.S01). **Supplemental information.** (Rinkus, 2/22/99).

123-208 165489 This consists of the following: 1) separate responses to issues discussed in worksheet W143945.831 regarding the analytical method; 2) an attachment containing typical chromatograms for the analyses of untreated feed samples; 3) an attachment containing handwritten data sheets and chromatograms for time-course studies of the loss of methyl bromide from dog feed after it had been fumigated; 4) an attachment containing handwritten data sheets and chromatograms for headspace analyses of methyl bromide after it had been spiked into polypropylene containers that were empty or that contained feed; and 5) an attachment containing the daily log sheets for the fumigation of the dog feed. This record is discussed in worksheet W143945.S01. **Supplemental information.** (Rinkus, 3/5/99).

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123-209 165490 This consists of the following: 1) a narrative that discusses the hematology and serum chemistry data in record 143945 as well as the newly submitted histological data for the thyroid and parathyroid contained in record 165490; 2) individual animal data sheets for all dogs on test regarding the microscopic examination of the thyroid and parathyroid glands; 3) historical control data from the conducting laboratory for the microscopic examination of the thyroid in 1-, 3- and 12-month studies; 4) the protocol for the study; and 5) protocol amendments for record 143945. This record is discussed in worksheet W143945.S01. **Supplemental information.** (Rinkus, 3/5/99).

048 913193(4110) "Chronic Ingestion by Dogs of Methyl Bromide Fumigated Food." (Albany Medical College, 1960) Methyl bromide fumigated food was fed to beagles, 4/group, daily at 0, 150, 75 or 35 mg/kg/day. **No adverse effect indicated:** Apparent NOEL = 75 mg/kg/day (lethargy, obesity, and one death at high dose). **Unacceptable.** Test article not characterized, no analysis of feed over the 6 to 8-week periods in which a given batch of test article was used, no necropsy/pathology data presented, too few animals (only 4 females at all treatment levels combined). J. Wong, 4-8-85.

123-161 132895 This record is an addendum to a letter from the Registrant to Jim Wells (director, DPR) dated October 19, 1994 (contained in the front of document 123-161). The letter and the addendum were submitted as a petition to DPR to drop its requirement for a dog inhalation chronic toxicity study. Record 132895 (and the letter) are reviewed in a memorandum from Dr. Rinkus to Dr. Gee dated January 19, 1995 (M950119). **Supplemental information. No worksheet.** (Rinkus, 7/24/95).

ACUTE/SUBACUTE, DOG

123-124 091578 "Acute Oral Toxicity Study in Beagle Dogs with Methyl Bromide," (Naas, D.; Wil Research Laboratories, Inc.; project no. WIL-49006; 10/9/90). Methyl bromide, 100% purity, was administered one time orally (corn-oil solutions in gelatin capsules) to beagle dogs (1/sex/treatment level) at 500, 50, 5, 3, and 1 mg/kg; no negative controls were used. Testing at 5 and 3 mg/kg consisted of using two different concentrations of methyl bromide: high concentration (HC), 158 and 138 mg/ml, respectively; and low concentration (LC), 63 and 64 mg/ml, respectively. Dogs were observed daily for clinical signs for 1-2 weeks postdosing, depending on the treatment level. Both dogs treated at 500 mg/kg exhibited severe signs of toxicity and vomiting and were found dead the next day; necropsy indicated toxicological effects in the stomach, kidneys, adrenal glands, and brain. No other dogs in the study died and no other dogs were necropsied. Severe signs of toxicity and vomiting of reddish material (presumably blood) were seen in the dogs treated at 50 mg/kg. The only other clinical sign seen in the other groups was vomiting, which in some cases contained reddish material. No vomiting was seen during the one week postdosing observation period in two dogs treated at 1 mg/kg or the females treated at 5 (LC) and 3 (HC) mg/kg. Supplemetary data. (Rinkus, 11/2/90).

123-124 091577 This record is a letter from the contract laboratory that conducted the acute oral dog study in record 091578 to Great Lakes Chemical Corp. (member company in the MBIP); it describes the observation of vomiting in two dogs treated once with methyl bromide at 5 mg/kg, using gelatin capsules that contained **microencapsulated** methyl bromide. Supplementary information. No worksheet. (Rinkus, 11/2/90).

123-163 132818 "An Up-and-Down Acute Inhalation Toxicity Study of Methyl Bromide in the Dog" (Newton, P.E.; Pharmaco LSR, Inc.; study number 93-6067; 9/14/94). One-day, two-day and four-day exposures were conducted as part of a range-finding process to select doses for an one-year exposure study. Dogs (one per concentration) were exposed for 7 h in the following order: 314 ppm, 233 ppm, 314 ppm, 394 ppm (6 h only due to severity of effects), 350 ppm and 345

ppm. Tremors and (or) trembling extremities were seen during exposure in each of the one-day experiments. **NOAEL (one day) = <233 ppm.** In the two-day exposure study, the six dogs used in the one-day exposure study were divided into two groups of three: one group was exposed to 268 ppm and the other, 283 ppm. At the start of this study, all dogs appeared clinically normal. The dogs were supposed to be exposed for four days (7 h/d) but the study had to be terminated after two days due to the observation of the following: severe neurotoxicity (delirium, thrashing and vocalization, tremors, traumatizing behavior [defined as slamming the head and body into the cage walls], depression, ataxia, irregular gait), rales and a cachectic appearance. Also, increased blood urea nitrogen and serum aspartate aminotransferase were serum chemistry findings for the dogs in both exposure groups. **NOAEL (two days) = <268 ppm.** The four-day exposure study used dogs that had not been exposed to methyl bromide previously. One male and one female were exposed to 55 ppm and 156 ppm for four days (7 h/d) and the dogs were terminated after the 4th exposure. Both dogs exposed to 156 ppm showed decreased activity during exposure on exposure days 3 and 4 and irregular gait during the postexposure observation period on exposure day 4. No abnormal signs were observed during or after exposure for the 55 ppm dogs. **NOAEL (four days) = 55 ppm.** Based on these results, the authors of record 132818 concluded that the cumulative effect for methyl bromide induced neurotoxicity made it difficult to estimate an exposure level which the dogs could tolerate for a 28-day or 1-year exposure study. **Supplemental information.** (Rinkus, 7/21/95).

123-164 132821 "A Four Week Inhalation Toxicity Study of Methyl Bromide in the Dog" (Newton, P.E.; Pharmaco LSR, Inc.; study no. 93-6068; 9/14/94). Methyl bromide (100% purity) was administered to beagle dogs (2-4 dogs per sex per treatment level) by whole body inhalation at 7 h/d, 5 d/week, for 23-24 exposure days (0, 25, 50 and 100 ppm), 30 exposure days (24 exposure days at 10 ppm, then 6 exposure days at 150 ppm), and 34 exposure days (0 and 5 ppm). Treatment levels were selected on the basis of a four-day exposure study (record 132818). Serum bromide levels were increased in a dose-response fashion in dogs exposed to $\geq$ 25 ppm. Bodyweight loss and neurotoxicity were seen in the dogs exposed to 150 ppm. Decreased activity was seen during exposure, starting on the 2nd exposure day to 150 ppm; and the dogs were in a poor condition during the final (6th) exposure. The next day three 150 ppm males had to be sacrificed due to exhibiting opisthotonos, irregular gait, opening and closing of the jaws and convulsions. The remaining 150 ppm dogs exhibited: nystagmus, intention tremors, ataxia, irregular gait and depression. Urinalysis indicated elevated levels of protein and bilirubin in the urine of the 150 ppm dogs. Histological examinations indicated that each of the 150 ppm dogs had cerebellar lesions (vacuoles in the granular layer) and olfactory degeneration; the males also had adrenal cortex findings (zona fasciulata, cytoplasmic vacuoles). Decreased bodyweight gain and less severe neurotoxicity (tremors, emesis, decreased activity during exposure but not postexposure) were seen in the 100 ppm dogs. One 100 ppm male exhibited a cerebellar lesion like that seen in the 150 ppm dogs. Decreased activity during exposures also was noted in two dogs exposed to 50 ppm, starting exposure day 14; but no findings were made for the 50 ppm group in postexposure examinations, including those done by a neurologist. **NOAEL (23-24 exposure days) = 50 ppm.** The female dogs exposed the longest to methyl bromide (5 ppm group) had reduced absolute spleen weight and two 5 ppm females were observed by the neurologist at the end of test week 6 to be less responsive than expected. Whether the latter constitutes an incipient neurological effect remains to be seen. **LOAEL (34 exposure days) = 5 ppm.** Major deficiencies include: inadequate conduct and reporting of the nervous system histological analysis (no in situ perfusion of brain; no musculature examination; possibly an inadequate number and selection of brain tissues examined); inadequate reporting of animal observations; and failure to secure organ weights, hematology, and serum chemistry data on the three 150 ppm males exhibiting the greatest neurotoxicity. **Supplemental information.** (Rinkus, 12/5/94).

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123-156 130781 This record is a letter dated June 8, 1994 from the Registrant to the Office of Pesticide Programs of USEPA, informing them that neurotoxicity had been observed in a 5-7 week dog inhalation study (record 132821). The letter indicates that the NOAEL was 100 ppm. The fact that DPR MT has set the NOAEL at 50 ppm is discussed in the rebuttal response of July 24, 1995 (R950724). **Supplemental information. No worksheet.** (Rinkus, 7/24/95).

ONCOGENICITY, RAT

Note: The one-liner for record 158746, a combined chronic toxicity-oncogenicity study using feed containing microcapsules of methyl bromide dissolved in corn oil, appears in the section "CHRONIC, RAT"

****084 059184** "Chronic (29-Month) Inhalation Toxicity and Carcinogenicity Study of Methyl Bromide in Rats," (Civo Institutes TNO, The Netherlands; report no. V86.469/221044, 1/87). Methyl Bromide, purity 98.8%, administered by whole body inhalation at concentrations of 0, 3, 30 or 90 ppm to 90 Wistar rats/sex per treatment level, 6 hours/day, 5 days/week for 29 months. Decreased bodyweight in the females and decreased survival in both sexes were observed in the high-dose groups. Nonneoplastic effects included: irritation of the epithelium of the nasal cavity (hyperplastic changes) in all treatment groups, decreased brain weight for high-dose females; and increased incidence of thrombi in the heart for both sexes in the high-dose groups. When first reviewed (Rinkus, 3/29/89), this study was considered unacceptable but upgradable upon submission of individual data and more information regarding the histological analyses of several organs (nasal cavity, thymus, hemopoietic system and brain). Individual data were submitted in record 116337 and historical control data were discussed in record 120402. Based on the brain weight data in record 116337, another adverse effect was identified: decreased absolute brain weight in both sexes surviving to terminal sacrifice, with a NOAEL of 3 ppm. In the second review (worksheet W059184.S01), the study was still considered unacceptable, but upgradable upon submission of histological data for the brains of rats in the interim sacrifice groups that died prematurely and other supplemental information regarding: the histological findings for the brain; the observation of neurological signs; and the historical control database (discussed in the rebuttal response R930205). The requested data were supplied in record 133417 and reviewed in worksheet W059184.S02. Since two neoplastic lesions from the 30 ppm female group originally diagnosed as gliomas had been reclassified as granular cell myoblastomas, the induction of gliomas was dropped as a possible adverse effect finding. Pursuant to the registrant's request, the setting of the LOAEL for olfactory-epithelium effects was revisited. I t was concluded that the LOAEL for the increased incidence of basal-cell hyperplasia in the olfactory epithelium was dependent on the duration of the exposure: for exposures lasting 12 months, 12-24 months and 24-29 months, the respective LOAELs were >90 ppm, 30 ppm and 3 ppm. Also, it was noted that degenerative changes (thinning of the overlying epithelium) accompanied the basal cell hyperplasia. Based on record 133417, record 059184 was upgraded to an **ACCEPTABLE** study (Rinkus, 7/10/95). In record 156300, the Registrant provided the results of a reexamination of the nasal-cavity histological slides from record 059184 by Drs. Jerry F. Hardisty (Experimental Pathology Laboratories, Inc.) and C.F. Kuper (TNO). Based on the reexamination, it was proposed that the LOAEL for olfactory epithelium effects be set at 30 ppm. However, this has not been accepted by DPR MT for reasons that include the following (discussed in worksheet W059184.S03): 1) the reexamination was not conducted in accordance with standard procedures for a peer review; and 2) even with the revised data, a dose response for incidence and severity was still evident, starting with the 3 ppm dose. (Rinkus, 9/23/97).

123-109 087806, 087807 IARC Monograph on methyl bromide (Vol. 41, pp. 187-212, 1986). No worksheet. (Rinkus, 3/2/90).

JJR 3/5/99

3/5/99

123-109 087798 Computer search of the IRIS data base on methyl bromide (bromomethane). No worksheet. (Rinkus, 6/4/90).

123-147 116337 This record contains the individual data for record 059184. It also contains organ-weight data for those rats from the main groups surviving to terminal sacrifice; these data were not mentioned in record 059184. **Supplemental information.** (Rinkus, 1/19/93).

123-148 120402 This record uses a question-and-answer format to address matters concerning record 059184 that were raised in the original review of this study (worksheet W059184.832) and in the rebuttal response R910712. The authors of this record are scientists at the Dutch-government laboratory that conducted the study reported in record 059184 (TNO-CIVO Toxicology and Nutrition Institute). **Supplemental information. No worksheet.** (Rinkus, 2/5/93).

123-148 120406 This record is a 2-page letter from Dr. Til of the TNO-CIVO Toxicology and Nutrition Institute to Dr. McAllister of Great Lakes Chemical Corporation. It contains corrections to the individual data (record 116337) and the original report (record 059184) that resulted from an audit of these records by the conducting laboratory. **Supplemental information. No worksheet.** (Rinkus, 2/5/93).

123-148 120408 This record is a photocopy of the first 9 pages of record 116337. **Supplemental information. No worksheet.** (Rinkus, 2/5/93).

123-166 133417 "Reevaluation of Pathology and Related Data Generated as Part of a Methyl Bromide Oncogenicity Study in Rats: Response to Questions Raised by the California Department of Food and Agriculture, Medical Toxicology Branch Document No. 123-147 (Addendum to Document 123-084)" (Bos-Kuijpers, M.H.M., Kuper, C.F., and Feron, V.J.; Civo Institutes TNO, The Netherlands; report no. V94.594; Nov., 1994). This record uses a question-and-answer format to address matters raised in R930205 concerning the histological, historical and neurological data contained in records 058194 and 116337. This record is discussed in worksheet W059184.S02. **Supplemental information. No worksheet.** (Rinkus, 7/24/95).

123-178 156300 "Chronic (29-Month) Inhalation Toxicity and Carcinogenicity Study of Methyl Bromide in Rats--Rexamination of Nasal Cavity" (J.F. Hardisty; Experimental Pathology Laboratories, Inc.; study number B-91-8213/002; July 21, 1997). At the request of the Registrant, Dr. Hardisty examined all nasal-cavity sections generated in the TNO rat inhalation study, record 059184. The intent was to determine "the accuracy and consistency of the initial diagnoses reported by the study pathologist," Dr. C.F. Kuper (TNO). All differences between the two pathologists were reconciled; i.e., agreement was reached on the final diagnoses in each case. Based on the reexamination, it was concluded that the LOAEL for the olfactory epithelium effects should be changed to 30 ppm. This record is reviewed in worksheet W059184.S03. **Supplemental information.** (Rinkus, 9/23/97).

123-174 143944 "Two-year Toxicological and Carcinogenesis Studies of Methyl Bromide in F344 Rats and BDF1 Mice" (Gotoh et al.; Japan Bioassay Laboratory; In: "Proceedings -- Second Asia-Pacific Symposium on Environmental and Occupational Health -- 1994", pp. 185-191). This is a 7-page report. It summarizes longterm studies done by inhalation (6 h/d, 5 d/w for 104 weeks) using F344/DuCrj rats and Crj:BDF1 mice; in both species, 50 animals per sex per dose were tested. Rats were exposed to 0, 4, 20 and 100 ppm; mice were exposed to 0, 4, 16 and 64 ppm. The authors indicated that there were no effects on survival in either species, that bodyweight reduction was mainly limited to the high-dose groups (both sexes) in both studies, that nonneoplastic effects were seen in the nasal cavity and cerebellum of the rats and mice, respectively, but that "evidence of carcinogenicity of methyl bromide was not obtained" in either species. However, inspection of the summary data indicates that the incidence of the following achieved statistical significance at the 0.01 level: pituitary adenoma in the 100 ppm male rats; adrenal-gland pheochromocytoma in the 4 ppm female rats; and liver adenoma in the 4 ppm female mice. Also, increased tumor inci-

dences in some methyl bromide-treated groups are a concern either due to the (presumed) rarity of the tumor (thyroid follicular-cell adenocarcinoma in the 100 ppm male rats; mesothelioma in the 20 ppm male rats) or the (apparent) failure to analyze tumor incidences for all sites combined (hemangioma/hemangiosarcoma in male mice; lymphoma in female mice). In order to do a complete evaluation of these studies, the full databases, including individual data, historical control data and subchronic studies, need to be submitted. **UNACCEPTABLE, UPGRADEABLE.** (Rinkus, 9/29/97).

ONCOGENICITY, MOUSE

****123-146 116243** "Toxicology and Carcinogenesis Studies of Methyl Bromide in B6C3F1 Mice (Inhalation Studies)," (Brookhaven National Laboratories, NTP Technical Report 385, March, 1992). Methyl bromide, purity 99.8%, was administered by whole body inhalation at concentrations of 0, 10, 33 and 100 ppm to 86 B6C3F1 mice/sex/treatment level, 6 h/day, 5 days/week for 2 years. Exposures to 100 ppm had to be stopped after 20 weeks due to debilitating neurotoxicity and mortalities, especially among the males; these groups (both sexes) were exposed only to untreated air for the remainder of the study. Treatment levels were chosen on the basis of subchronic testing which was included in the report. Interim sacrifices of ~10 mice/sex/treatment level were performed at 6 months and 15 months; also, 16 mice/sex/treatment level primarily were used for neurobehavioral testing every 3 months. Clinical signs indicative of neurotoxicity (tremors, paralysis, unusual gait, abnormal posture) were observed in 78% of the males and 43% of the females exposed to 100 ppm methyl bromide and were observed in 2-3% of the mice (both sexes) exposed to 33 ppm methyl bromide. In many cases, the clinical signs in the 100 ppm groups began to appear well after their exposure to methyl bromide had stopped. Neurobehavioral testing identified effects in the 100 ppm groups at 3 months (both sexes) and later (only females could be tested). Neurobehavioral testing also found effects in the 10 ppm and 33 ppm groups starting after 6 months of exposures. Decreased bodyweight was observed in the 33 ppm and 100 ppm female groups and the 100 ppm male group. Heart lesions, either cardiac degeneration or chronic cardiomyopathy, were observed in 80% of the males and 69% of the females exposed to 100 ppm methyl bromide; also, the incidence of chronic cardiomyopathy in the male 33 ppm group (20%) was greater than that seen in the controls (8%). Sternal dysplasia was observed at low incidence (4-6%) in the 10 ppm and 33 ppm female groups and the 33 ppm male group and was observed at a higher incidence (15-20%) in the 100 ppm groups (both sexes). The rarity and the late onset for the sternal lesion raises the possibility that it is the result of some type of neuromuscular toxici-ty, as opposed to a direct effect on the sternum. Degenerative lesions in the brain were observed in 44% and 18% of the male and female 100 ppm groups, respectively. The lesions were located in the cerebellum (internal granular layer cells) and sometimes were accompanied by degenera-tive lesions in the cerebrum. Since some brain lesions were seen in 100 ppm mice surviving till terminal sacrifice (therefore not exposed to methyl bromide since test week 20), some damage caused by methyl bromide to the brain is not repairable. Olfactory epithelium lesions, either necrosis or metaplasia, were observed in 12% of the mice exposed to 100 ppm methyl bromide (both sexes). **NOAEL < 10 ppm (neurobehavioral testing changes, sternal dysplasia).** No evidence of any carcinogenicity was observed. This study is considered **ACCEPTABLE.** (Rinkus, 11/6/92).

123-145 076659 This record is an exact duplicate of record 116243. **Supplemental information. No worksheet.** (Rinkus, 7/24/95).

123-174 143944 "Two-year Toxicological and Carcinogenesis Studies of Methyl Bromide in F344 Rats and BDF1 Mice" (Gotoh et al.; Japan Bioassay Laboratory; In: "Proceedings -- Second Asia-Pacific Symposium on Environmental and Occupational Health -- 1994", pp. 185-191). This is a 7-page report. It summarizes longterm studies done by inhalation (6 h/d, 5 d/w for 104 weeks) using F344/DuCrj rats and Crj:BDF1 mice; in both species, 50 animals per sex per dose were tested. Rats were exposed to 0, 4, 20 and 100 ppm; mice were exposed to 0, 4, 16 and 64 ppm. The au-

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thors indicated that there were no effects on survival in either species, that bodyweight reduction was mainly limited to the high-dose groups (both sexes) in both studies, that nonneoplastic effects were seen in the nasal cavity and cerebellum of the rats and mice, respectively, but that "evidence of carcinogenicity of methyl bromide was not obtained" in either species. However, inspection of the summary data indicates that the incidence of the following achieved statistical significance at the 0.01 level: pituitary adenoma in the 100 ppm male rats; adrenal-gland pheochromocytoma in the 4 ppm female rats; and liver adenoma in the 4 ppm female mice. Also, increased tumor incidences in some methyl bromide-treated groups are a concern either due to the (presumed) rarity of the tumor (thyroid follicular-cell adenocarcinoma in the 100 ppm male rats; mesothelioma in the 20 ppm male rats) or the (apparent) failure to analyze tumor incidences for all sites combined (hemangioma/hemangiosarcoma in male mice; lymphoma in female mice). In order to do a complete evaluation of these studies, the full databases, including individual data, historical control data and subchronic studies, need to be submitted. **UNACCEPTABLE, UPGRADEABLE.** (Rinkus, 9/29/97).

REPRODUCTION, RAT

****123-082 058196** "Two-Generation Reproduction Study Via Inhalation in Albino Rats Using Methyl Bromide," (American Biogenics Corporation, Decatur, IL; laboratory study number 450-1525, 2/19/86). Methyl Bromide (lot and purity not stated) was administered to Sprague Dawley rats of both sexes by whole body inhalation 6 h/day for 5 days/week at the nominal levels of 0, 3, 30 or 90 ppm. Parental animals were exposed for about 40 or 55 days and 90-105 days before their first and second matings, respectively, and were exposed for a total of 132-145 days before they were sacrificed. Premating bodyweights were decreased statistically only in F0 males in the 90 ppm group. Absolute brain weights were decreased in F0 males, F1 males, and F0 females in the 90 ppm groups. In the second mating of the F1 parents, the fertility index decreased from 90.9% in the controls to $\leq$ 68% in the 30 and 90 ppm groups. The progeny from the 30 and 90 ppm groups exhibited statistically reduced bodyweights at weaning in each of the four litters produced by these groups. For the female F2b progeny from the 90 ppm group, the absolute weights of the brain, heart, kidneys, and liver were reduced statistically; weight reductions of a lesser degree also occurred for the kidneys, liver, and testes of the corresponding male progeny. When first reviewed (3/21/89), the parental NOEL was tentatively set at 3 ppm based on the reduced fertility seen at 30 ppm and the study was considered unacceptable but upgradeable upon submission of: 1) lot number and purity of test article; 2) more details about exposure conditions and monitoring; and 3) microscopic examination of target organs in parents per EPA guidelines. Items 1 and 2 were satisfied by the submission of DPR documents 123-109 (attachment 6 [no record number]) and 123-139, respectively. Item 3 was marginally satisfied by the submission of record 125516. Items 1-3 are discussed in worksheet W058196.S01. The quantitative histological data indicate that in the F1 90 ppm groups (both sexes), there was a decrease in the width of the cerebral cortex (section III-h in the sectioning scheme of Rodier and Gramann [Neurobehavioral Toxicology, 1:129-135, 1979]). Other parameters also were decreased in the F1 90 ppm females (parameters IIh and IVb) or F1 males (parameters IIIa and IIId). Since the mid- and low-dose F1 groups have not been examined, no NOAEL has been established for these effects per se. However, the reduced brain weights for the F1 30 ppm females will be used to assume that the LOAEL for the reduced cerebral-cortex width is 30 ppm. Quantitative histological parameters were not affected in the F0 90 ppm adults, thus indicating that the F1 effects were the result of the perinatal exposure of the F1 rats. No gliosis or other brain lesions were noted in any F1 or F0 adults. **Parental NOAEL = 3 ppm (reduced fertility). Progeny NOAEL = 3 ppm (decreased pup bodyweight and some organ weights; reduced F1 brain weight/reduced width of the cerebral cortex).** It should be noted that the pregnant dams in this study were only exposed 5 d/w (for a total of 14-15 d) during their pregnancy and that the pups were not directly exposed until after weaning (postnatal day 28). This study is now considered marginally **ACCEPTABLE**. (Rinkus, 5/26/95).

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094 059912 Protocol to 082 058916. No worksheet; not reviewed. (Kishiyama, 3/21/89).

123-139 111505 This record concerns the analytical measurements of the methyl bromide atmospheres generated in record 058196. This record is discussed in worksheet W058196.S01. **Supplemental information. No worksheet.** (Rinkus, 7/24/95).

123-142 113606 "Histopathological Evaluation of Brains from Rats--Inhalation Study of Methyl Bromide," (Hardisty, J.F.; Experimental Pathology Laboratories, Inc., Research Triangle Park, NC; EPL Project Number: 303-007; 3/2/92). This record contains qualitative histological data from the analyses of the brains of the F1 adults generated in record 058196. This record is discussed in worksheet W058196.S01. **Supplemental information. No worksheet.** (Rinkus, 7/24/95).

123-153 125516 "Chemical Manufacturer's Association Study Number 450-1525: Neuropathological Evaluation of Brains from F0 and F1 Rats in a Two-Generation Reproduction Study with Methyl Bromide--Pathology Report" (W. M. Busey; Experimental Pathology Laboratories, Inc.; EPL project number 303-007; Feb. 25, 1993). This record contains quantitative histological data for the brains of the F1 and F0 adults exposed to 0 ppm or 90 ppm (only dose levels considered) from record 058196. These data indicate that in the F1 90 ppm groups (both sexes), there was a decrease in the width of the cerebral cortex (section III-h in the sectioning scheme of Rodier and Gramann [Neurobehavioral Toxicology, 1:129-135, 1979]). Other parameters also were decreased in the F1 90 ppm females (parameters IIh and IVb) or F1 males (parameters IIIa and IIId). Quantitative histological parameters were not affected in the F0 90 ppm adults, thus indicating that the F1 effects were the result of the perinatal exposure of the F1 rats. This record was reviewed in worksheet W058196.S01. **Supplemental information. No worksheet.** (Rinkus, 7/24/95).

123-152 124863 This record is an unsigned, "draft" version of record 125516. This record has not been reviewed since it was superseded by record 125516. **Supplemental information. No worksheet.** (Rinkus, 7/24/95).

No Record Number. "Protocol for the Neuropathological Evaluation of Brains from F0 and F1 Rats in a Two-Generation Reproduction Study with Methyl Bromide, Toxigenics Study Number 450-1525," (W. M. Busey; Experimental Pathology Laboratories, Inc.; May 8, 1992). This protocol, which was sent by fax as a response to a telephone conference between the representatives of the MBIP and DPR MT on May 5, 1992, is the protocol for record 125516. This protocol was discussed in the rebuttal response of May 13, 1992 (R920513). **Supplemental information. No worksheet.** (Rinkus, 7/24/95).

123-109 087804 "Evaluation of Spermatogenesis and Sperm Quality in the Rat Following Acute Inhalation Exposure to Methyl Bromide," (Hurtt, M.E. & Working, P.K., Fund. Applied Toxicol. 10: 490-498, 1988). Methyl bromide (99.9% pure) was given by inhalation to adult male Fischer 344 rats at 0 (air) or 200 ppm for 6 h/day for 5 days. Rats from both treatment groups were sacrificed (5 or 10 per group, depending on the day) at the following times: days 1 (first day of exposure), 3, 5, 6, 8, 10, 17, 24, 38, 52, and 73. At day 5, the methyl bromide-treated group weighed ~10% less than the control group and continued to weigh less till day 52. The methyl bromide group exhibited lower plasma testosterone on days 1, 3, 5, and 6 and and a decrease in nonprotein sulfhydryl in the testis and liver on days 1 and 3. Endpoints that were not affected were: clinical signs; testis weight; testicular and epididymal histology; daily sperm production; cauda epididymal sperm count; sperm morphology; sperm motility; and linear sperm velocity. However, CDFA notes spermatocytes and differentiating spermatogonia were sampled only once each (days 52 and 73, respectively); this could be important for sperm parameters like sperm count, morpholoIgy, and motility. The authors compared these test results with those seen in rats inhaling 3000+ ppm methyl chloride in a similar acute exposure. Supplemental information. No worksheet. (Rinkus, 2/26/90).

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TERATOGENICITY, RAT

**123-039 026866 "Teratologic Assessment of Butylene Oxide, Styrene Oxide and Methyl Bromide (Rats)" (Battelle, Pacific Northwest Laboratory, contract no. 210-78-0025; NIOSH Technical Report, July 1981). Pure methyl bromide was administered to Wistar rats by whole body inhalation 7 hrs/day on days 1 to 19 of gestation at 0, 20 or 70 ppm. Some groups received pregestational exposure for 5 days/week over three weeks immediately prior to mating. The following combinations of pre- and post-mating treatments were employed:0/0, 0/20, 0/70, 20/0, 20/20, 70/0, and 70/70 ppm pre/post-treatment. Initially reviewed as: no apparent adverse effects indicated; maternal NOEL = 20 ppm (diminished body weight gain in early to mid gestation); apparent developmental NOEL = 20 ppm (treatment-related skeletal and delayed ossification effects); unacceptable, upgrade possible; J. Remsen (Gee), 9-4-85; C. Aldous, 10/20/87. In the second review by Rinkus (4/13/89), it was concluded that the high dose did not obviously affect dam bodyweights; maternal NOEL was revised to: > 70 ppm and developmental NOEL remained 20 ppm. The study was considered unacceptable, but upgradeable upon submission of: evidence that test material was technical grade; evidence that a MTD essentially was tested; and individual data for mothers and fetuses. The study is now considered ACCEPTABLE because: technical grade material typically is of high purity like that used in this study; while 70 ppm probably is less than half of a MTD, this is a moot point since the high-dose did exert an effect (delayed skull ossification); and the review of the individual data to see if the effect is being mediated by maternal toxicity will be done if necessary in the risk assessment phase. (Rinkus, 5/24/91).

NOTE: The memo from EPA to CDFA addressing differences in data gap status for this chemical (dated 2/17/89) notes EPA classification as having been changed from "Core Minimum" to "Core Supplementary" (but upgradeable).

092 059690 Partial duplicate to 039 026866. No worksheet. (Kishiyama, Rinkus, 4/13/89)

039 026867 "Teratogenicity Investigation of Orally Administered Methyl Bromide." (An investigation "conducted by Dutch authorities" translated for EPA by Great Lakes Chemical Company, 6-81) Methyl bromide, no purity given, was administered to rats by gavage on day 5 to 20 of gestation at 0 (peanut oil), 0.5, 5, 25 or 50 mg/kg. **Unacceptable.** Poor translation, incomplete with no data. J. Remsen (Gee), 9-4-85.

NOTE: This study was not available to EPA for review as of 2/17/89.

No Record Number. "Oral Teratogenicity Studies of Methyl Bromide in Rats and Rabbits" (Kaneda, M, Hojo, H., Teramoto, S. & Maita, K.; Institute of Environmental Toxicology, Tokyo, Japan; Food Chem. Toxicol. 36:421-427, 1998). Methyl bromide (purity 99.5%) dissolved in corn oil was administered by gavage to 23-24 pregnant Crj:CD (SD) rats/dose at 0 (corn oil), 3, 10 and 30 mg/kg on gestation days 6-15 and to 15-18 pregnant Kbl:JW rabbits at 0 (corn oil), 1, 3 and 10 mg/kg on gestation days 6-18. Rats and rabbits were sacrificed on gestation days 20 (ether inhalation) and 27 (pentobarbital iv injection), respectively. The highest doses tested were selected (apparently) on the basis of preliminary studies that included dosing rats and rabbits at 25 and 30 mg/kg, respectively. The dosing volumes were 10 mL/kg for rats and 0.5 mL/kg for rabbits; as a result, the high-dose rats were gavaged with a 3 mg/ml solution while the high-dose rabbits were gavaged with a 20 mg/mL solution. In both species, maternal effects were observed only in the high-dose groups. Both species exhibited decreased bodyweight gain; but only rabbits lost bodyweight relative to predosing. Decreased food consumption occurred in both species; in the case of the rats, the fact that the negative control group also exhibited decreased food consumption suggests that the large volume of corn oil used (10 mL/kg) or the act of being gavaged constituted a stress on the animals. At necropsy, only the high-dose rats had findings: all dams exhibited erosion and thickening of the wall in the nonglandular part of the stomach and adhesions between the stomach and the spleen, liver or diaphragm. In both species, no clinical signs were observed (i.e., no neurotoxicity). In rats, the only fetal findings of interest were seen in the high-dose group: microphthalmia in two fetuses (two litters [8% incidence]) and having 25 (not 26) presacral vertebrae in five fetuses (two litters [8% incidence]); no cases of microphthalmia or decreased verte-

brae count were seen in the negative-control group. While neither effect was statistically significant, typically both of these findings are seen infrequently in negative-control litters using Sprague Dawley rats (i.e., ≤ 1% litter incidence). In rabbits, total litter resorption occurred with two high-dose does and one negative-control doe; the number of resorptions involved in these instances was not indicated. In rabbits, the only fetal finding of interest was the observation that each of the three methyl bromide-treated groups had more fetuses with skeletal malformations than what was observed in the negative-control group. Skeletal malformations involving 2-3 litters in at least one methyl bromide-treated group included: splitting of the nasal/frontal/parietal bones; hemivertebra; fusion of the ribs/sternebrae; and absence of the metacarpal and phalangeal bones. At the litter level, no increased incidence was statistically significant nor were there any dose responses. Notwithstanding that historical negative-control data for Kbl:JW rabbits are not generally available in the open literature, the differences between the negative-control and methyl bromide-treated groups appear too small to warrant further concern. **Supplemental information.** (Rinkus, 12/23/98).

TERATOGENICITY, RABBIT

039 026865 "Teratologic Assessment of Butylene Oxide, Styrene Oxide and Methyl Bromide - Rabbits." (NIOSH, 9-82) Methyl bromide, 99.5%, was administered by whole body inhalation to New Zealand White rabbits, 7 hrs/day, day 1 to 24 of gestation at 0, 20 or 70 ppm, 24/group. **Unacceptable.** No individual data, 2 doses only with one too high. J. Remsen (Gee), 9-4-85. It should be noted that neurotoxicity and death were observed in the rabbits inhaling 70 ppm methyl bromide in this study. The onset of the neurotoxicity and death occurred concurrently after about 1 week of exposures. Out of a group of 25 does, 3 were dead by gestation day 10, increasing to a total of 9 dead by gestation day 15, when exposures were stopped; all does in this group except one were dead by gestation day 30. (Rinkus, 1/17/92).
NOTE: EPA did not accept this study for regulatory purposes (see EPA Re-registration Guidance document of Aug., 1986, 123-071, p. 9).

092 059690 Partial duplicate to 039 026865. No worksheet. (Kishiyama, Rinkus, 4/13/89)

104 066800 Protocol (draft). A letter from Hazleton Laboratories dated January 28, 1988 for a rabbit teratology study indicates a final protocol is pending. No worksheet. (Kishiyama, 1/24/89)

****123-127 095930** "Methyl Bromide Inhalation Teratology Study in New Zealand White Rabbits," (Breslin et al.; The Toxicology Research Laboratory, Dow Chemical Company; Laboratory Project Study ID number K-000681-033; 6/18/90). Methyl bromide was administered by whole body inhalation 6 h/d on days 7-19 of gestation at concentrations of 0, 20, 40 and 80 ppm to 15-21 pregnant New Zealand White rabbits/treatment level (part I) or 0 and 80 ppm to 15-16 pregnant does/treatment level (part II); does were sacrificed on day 28. Treatment levels were chosen on the basis of a pilot study, which is now on file at CDPR (record 111266). Maternal effects were limited to the 80 ppm groups and consisted of decreased bodyweight gains and clinical signs indicative of neurotoxicity (part I only, 3 does: right-sided head tilt, ataxia, slight lateral recumbency, lethargy). **Maternal NOAEL = 40 ppm (neurotoxicity).** Fetal bodyweight was decreased statistically in the 80 ppm group in part II. Fetal effects that appeared to be the results of treatments included: omphal-ocele (80 ppm group, part I); hemorrhaging with or without hydrops (80 ppm, parts I & II); retroesophageal right subclavian artery (80 ppm group, part I); gall bladder agenesis (80 ppm, parts I & II); and fused sternebrae (80 ppm, part I; no skeletal analysis in part II). When first reviewed (5/3/91), this study was considered **UNACCEPTABLE**, with a developmental NOAEL of 20 ppm (fused sternebrae; omphalocele); and to upgrade the following had been requested: 1) necropsy data of pups/fetuses of 80 ppm does that delivered early or were found dead; 2) the pilot study; and 3) clarification of matters concerning historical control data, umbilical hernia/omphalocele & number

bred in part II. These data have now been submitted (records 111265 and 111266) and, as discussed in worksheet W095930.S01, the matters that they address are now considered resolved. **Developmental NOAEL = 40 ppm (omphalocele, hemorrhaging with or without hydrops, retroesophageal right subclavian artery, gall bladder agenesis, fused sternebrae and decreased fetal bodyweight).** This study now is considered **ACCEPTABLE.** (Rinkus, 1/15/92).

123-137 111265 This record contains the following supplementary information to record 095930: individual responses to the matters raised in W095930.833; the protocol to record 095930; raw da-ta regarding animal observations and (or) the gross pathology examination of two 80 ppm does which either delivered early or was found dead; a table identifying the route of administration used in the studies that comprise the historical control database for the conducting labortory; an updated version of this historical control database; and some text regarding the management of mucoid enteritis in rabbits. Discussion of this record is contained in the worksheet W095930.S01 **Supplementary information. No worksheet.** (Rinkus, 1/16/92).

123-138 111266 "Methyl Bromide Inhalation Teratology Probe Study in New Zealand White Rabbits," (Breslin et al.; The Toxicology Research Laboratory, Dow Chemical Company; Laboratory Project Study ID numbers K-000681-032 & K-000681-032A; 4/2/90). This study was not a teratology study; rather, it was designed only to evaluate maternal toxicity and embryolethality so that the high dose in a standard teratology study (record 095930) could be set; also histological examinations of the brain (parts I & II) and spinal cord (part II) were performed. Methyl bromide was administered by whole-body inhalation 6 h/d on days 7-19 of gestation at concentrations of 0, 10, 30, and 50 ppm to 4-7 pregnant New Zealand White rabbits/treatment level (part I) or 0, 50, 70, and 140 ppm to 6-7 pregnant does/treatment level (part II). Does were sacrificed on day 20, with the exception of the 140 ppm group: these does were sacrificed on day 17 (i.e., after 10 exposure days) due to their moribund state. Clear maternal effects were limited to the 140 ppm group and included: decreased bodyweights and bodyweight gains and clinical signs of neurotoxicity (lethargy, labored breathing, ataxia, right-sided head tilt, reduced sensations in the extremities, dilated pupils, lateral recumbency, loss of placing or righting reflex, and rear leg splay). Histological examinations of the brains of all does on test indicated that only the 140 ppm group had pathological lesions (multifocal areas of inflammation of the meninges overlying most regions of the brain and/or bilaterally symmetrical necrosis or spongiosis of the midbrain dorsolateral to the pyramidal tracts). Fetal examinations were limited to counting the number of implantations and resorptions. A reduction in litter size for the 70 ppm group in association with an increase in preimplantation loss was suggested by the data (no evaluation of 140 ppm group was provided). The authors noted that these effects were not observed again in the full study (record 095930). **Supplemental information. No worksheet.** (Rinkus, 1/16/92).

123-141 112841 This record contains the protocol for record 095930; this protocol also is found in record 111265. **No worksheet.** (Rinkus, 2/5/93).

123-141 (no record number) The front of this document contains a follow-up response dated 1/9/92 by Dr. Breslin regarding his responses contained in record 111265 regarding 095930. It addresses: why animal identification numbers were noncontinuous; the number of uteri stained with sodium sulfide; and corrections in the historical control data regarding frequency of umbilical hernia. **No worksheet.** (Rinkus, 2/5/93).

No Record Number. "Oral Teratogenicity Studies of Methyl Bromide in Rats and Rabbits" (Kaneda, M, Hojo, H., Teramoto, S. & Maita, K.; Institute of Environmental Toxicology, Tokyo, Japan; Food Chem. Toxicol. 36:421-427, 1998). Methyl bromide (purity 99.5%) dissolved in corn oil was administered by gavage to 23-24 pregnant Crj:CD (SD) rats/dose at 0 (corn oil), 3, 10 and 30 mg/kg on gestation days 6-15 and to 15-18 pregnant Kbl:JW rabbits at 0 (corn oil), 1, 3 and 10 mg/kg on gestation days 6-18. Rats and rabbits were sacrificed on gestation days 20 (ether inhalation) and 27 (pentobarbital iv injection), respectively. The highest doses tested were selected (apparently) on the basis of preliminary studies that included dosing rats and rabbits at 25 and 30

MR 2/6/99 220 2/6/99

mg/kg, respectively. The dosing volumes were 10 mL/kg for rats and 0.5 mL/kg for rabbits; as a result, the high-dose rats were gavaged with a 3 mg/ml solution while the high-dose rabbits were gavaged with a 20 mg/mL solution. In both species, maternal effects were observed only in the high-dose groups. Both species exhibited decreased bodyweight gain; but only rabbits lost bodyweight relative to predosing. Decreased food consumption occurred in both species; in the case of the rats, the fact that the negative control group also exhibited decreased food consumption suggests that the large volume of corn oil used (10 mL/kg) or the act of being gavaged constituted a stress on the animals. At necropsy, only the high-dose rats had findings: all dams exhibited erosion and thickening of the wall in the nonglandular part of the stomach and adhesions between the stomach and the spleen, liver or diaphragm. In both species, no clinical signs were observed (i.e., no neurotoxicity). In rats, the only fetal findings of interest were seen in the high-dose group: microphthalmia in two fetuses (two litters [8% incidence]) and having 25 (not 26) presacral vertebrae in five fetuses (two litters [8% incidence]); no cases of microphthalmia or decreased vertebrae count were seen in the negative-control group. While neither effect was statistically significant, typically both of these findings are seen infrequently in negative-control litters using Sprague Dawley rats (i.e., $\leq$ 1% litter incidence). In rabbits, total litter resorption occurred with two high-dose does and one negative-control doe; the number of resorptions involved in these instances was not indicated. In rabbits, the only fetal finding of interest was the observation that each of the three methyl bromide-treated groups had more fetuses with skeletal malformations than what was observed in the negative-control group. Skeletal malformations involving 2-3 litters in at least one methyl bromide-treated group included: splitting of the nasal/frontal/parietal bones; hemivertebra; fusion of the ribs/sternebrae; and absence of the metacarpal and phalangeal bones. At the litter level, no increased incidence was statistically significant nor were there any dose responses. Notwithstanding that historical negative-control data for Kbl:JW rabbits are not generally available in the open literature, the differences between the negative-control and methyl bromide-treated groups appear too small to warrant further concern. **Supplemental information.** (Rinkus, 12/23/98).

GENE MUTATION

Note: Document 123-109 contains various published reports regarding the mutagenic potential of methyl bromide. In each case, the experimental details for the mutagenicity testing were not reported adequately, which is often observed with reports published in the open literature. Inadequate documentation of methods is viewed by CDFA as a significant reason for officially rejecting a study. However, CDFA also recognizes that these studies collectively indicate that methyl bromide is a direct-acting mutagen. Since this opinion now is endorsed by the Sponsor also (see Attachment 1 in document 123-109), these studies have been considered collectively as satisfying this data requirement, despite their individual shortcomings. (Rinkus, 2/23/90).

103 066722 "Sex-Linked Recessive Lethal Test in Drosophila Melanogaster," (Inveresk Research International Ltd., Scotland; report no. 1190, 5/30/81). Two separate stocks of wild-type male fruit flies (D. melanogaster; Oregon K) were exposed to air containing either 20 or 70 ppm of test material for 5 h and subsequently were mated to Muller-5 females to produce F1 females, which were mated to produce the F2 progeny in which the frequency of lethal mutations was scored (Muller-5 test). Treatments with test material did not produce any signs of toxicity or affect fertility. An increased frequency of lethals that was observed for the 20 ppm group using one stock of males was not similarly observed in the corresponding group of the second stock of males nor in either stocks treated at the 70 ppm level with test material. UNACCEPTABLE and not upgradable because testing up to a MTD clearly was not achieved and the testing failed in other ways to meet the EPA guidelines for this assay. (Kishiyama, 2/2/89; Rinkus, 4/6/89).

SAR 3/5/99 221 3/5/99

123-109 087801 "Mutagenic Activity of Chemicals Identified in Drinking Water," (Simmon et al., In: Progress in Genetic Toxicology, Scott et al. (Eds.), pp. 249-258, Elsevier/North Holland Biomedical Press, 1977). Methyl bromide (purity not stated) was tested in the Ames test using TA100; testing did not involve the use of any metabolic activation system like S-9. The experimental details were not described adequately. Agar plates containing bacteria were incubated for 21 h at 37°C in 9-liter dessicators that contained methyl bromide concentrations of 0 (air), 0.01, 0.02, 0.05, 0.10, and 0.20 % (i.e., 0, 100, 200, 500, 1000, and 2000 ppm). Stirring bars were used as fans to achieve an even distribution of vapors, but the number of plates per dessicator was not stated. A doubling in the spontaneous number of revertants was seen at the lowest concentration tested; and the number of revertants continued to increase with increasing concentration, up to a maximun effect at the 0.1% treatment level. **UNACCEPTABLE.** No worksheet. (Rinkus, 2/23/90).

123-109 087802 "Mutagenicity of Methyl Bromide in a Series of Short-Term Tests," (Kramers et al., Mutation Res. 155: 41-47, 1985). Methyl bromide of 99% purity was tested for genotoxicity in the following assays: a fluctuation test using Klebsiella pneumoniae; the Ames test using Salmonella typhimurium strains TA100 and TA98; the induction of forward mutations at the TK locus and at the HGPRT locus using L5178Y mouse lymphoma cells; the induction of unscheduled DNA synthesis (UDS) using freshly isolated rat liver cells; and the induction of sex-linked recessive lethal mutations using Drosophila melanogaster. The experimental details were not described adequately. Exposures to methyl bromide were accomplished by: exposing the tester organisms to vapors formed in closed containers into which an ethanolic solution had been introduced (fluctuation test, Ames test); adding an ethanolic solution directly to gas-tight bottles ~90% filled with cell media (mouse lymphoma assay, UDS assay); or exposing the tester organisms in a chamber to a continuous flow of methyl bromide-containing atmospheres (Drosophila). Methyl bromide was active in all tests, except the UDS testing. Lowest treatments that exhibited a positive effect were: 1) fluctuation test, 4750 mg/m ^{3} (1271 ppm; the estimated concentration of methyl bromide in the nutrient broth was 250 μM); 2) TA100, 1900 mg/m ^{3} (508 ppm) (no mutagenicity seen with TA98); 3) L5178Y cells, ~0.3 μM; and Drosophila, 3 weeks of 6 h/day, 5 day/week using 200 mg/m ^{3} (52 ppm). UDS testing conducted up to a maximum concentration of 0.3 mM did not detect an effect, but it was not stated whether the HDT was sufficient to cause cytotoxicity. **UNACCEPTABLE**. No worksheet. (Rinkus, 2/23/90).

123-109 087803 Abstract to work discussed in record 087802. No worksheet. (Rinkus, 2/26/90).

123-109 087808 "Further Mutagenicity Studies on Pesticides in Bacterial Reversion Assay Systems," (Moriya et al., Mutation Res. 116: 185-216, 1983). Methyl bromide (purity not stated) was tested for mutagenicity using the Salmonella typhimurium strains TA100, TA1535, TA1537, TA1538, and TA98 and the Escherichia coli strain WP2 hcr. Experimental details were not report-ed adequately. Testing involved placing one bacteria-containing agar plate without its lid upside down in a glass container, injecting gaseous methyl bromide into the container, and incubating for 2 days at 37°C while an electric fan stirred the atmosphere in the container. The lowest test concentration to increase the revertant frequency of TA100 was ~500 mg/m ^{3} (134 ppm). Other strains listed as showing a positive response were: TA1535 and WP2 hcr. It was stated without data that the mutagenicity of methyl bromide was not greatly affected by the use of a S-9 mix. This study also indicates that chloropicrin, which is often combined with methyl bromide in formulated fumi-gant products, was mutagenic in WP2 hcr and TA98 in the absence of S-9 and in TA100 in the presence of S-9; the chloropicrin testing involved the standard plate assay. **UNACCEPTABLE**. No worksheet. (Rinkus, 3/2/90).

123-109 087809 "Estimation of Genetic Risks of Alkylating Agents. VI. Exposure of Mice and Bacteria to Methyl Bromide," (Djalahi-Behzad et al., Mutation Res. 84: 1-9, 1981). Methyl bromide (purity not stated) was tested for mutagenicity using Escherichia coli Sd-4, but the experimental details were not reported adequately. Also, adduct formation of methyl bromide with hemoglobin and DNA in test-tube reactions and in mice exposed to methyl bromide by either inhalation or by intraperitoneal injection was determined. Inhalation exposure involved the use of a static system

MP 2/6/99 222 2/1/99

in which 9 mice in an 11-liter chamber inhaled an atmosphere for 4 h that initially contained 36 or 17 ppm (CDFA calculation of ppm concentration). Intraperitoneal exposure involved the single injection of a corn-oil solution to give a dose of 417 μg/kg bodyweight. Bacterial mutagenicity was observed at test concentrations of $\geq$ 4 mM; the LD50 for these test conditions was 6-8 mM. N-7-methylguanine formation was 10 times greater in DNA isolated from the spleen than that measured in the liver (only organs sampled) of mice inhaling the high dose; DNA adduct formation was not assayed for the low inhalation dose or for the intraperitoneal exposure. Protein alkylation was 22 times greater in RBCs than in the liver for mice inhaling the high dose; protein alkylation was also measured at the low inhalation dose and in the intraperitoneal experiment. **UNACCEPTABLE.** No worksheet. (Rinkus, 3/8/90).

123-146 116243 "Toxicology and Carcinogenesis Studies of Methyl Bromide in B6C3F1 Mice (Inhalation Studies)--Ames Test," (National Toxicology Program Technical Report 385; March, 1992). As part of the National Toxicology Program, the mouse inhalation cancer bioassay, record 116243, also contained data for mutagenicity testing using the Ames test. Testing was performed using dessicators into which methyl bromide/air mixtures were introduced. These data indicated a positive and reproducible response. However, the supposed lowest test concentration, 0.004 moles per liter, would be equivalent to a methyl bromide atmosphere of 100,000 ppm and such a concentration should be much too high to allow for any survival. Possibly, the reporting of the test concentrations is a typographical error. **UNACCEPTABLE.** No worksheet. (Rinkus, 2/5/93).

CHROMOSOME EFFECTS

Note: EPA is requiring both bone marrow and sister chromatid exchange tests (see EPA Re-registration Guidance document of Aug., 1986).

044 035750 [Previous Record # = 913095-1] "Effect of Methyl Bromide on the Frequency of Sister Chromatid Exchanges (SCE) in Chinese Hamster Ovary (CHO) Cells." (Pasadena Foundation for Medical Research, 1980) Methyl bromide, purity not given, was assayed with Chinese Hamster Ovary cells at 0, 1, 6, 13 or 26 ppm for SCEs. **Possible adverse effect**: dose-related increase in SCEs. **Unacceptable.** Protocol not provided, criteria for scoring SCEs not provided. J. Wong, 4-8-85. [There is no apparent merit in seeking to "upgrade" this study, as EPA is requiring additional studies of this type in any case].

103 066721 "Cytogenetic Analysis of Rat Bone Marrow Cells," (Inveresk Research International Ltd., Scotland; report no. 1190, 5/30/81). Methyl bromide was administered by whole body inhalation at concentrations of 0 (air), 20 and 70 ppm to Sprague Dawley rats of both sexes. One group of 30 rats/treatment level received only one 7-h exposure and another group of 10 rats/treatment level received 5 consecutive daily exposures of 7 h/day. The former were sampled at 6, 24 and 48 hours posttreatment whereas the latter were sampled 6 hours posttreatment. There was no obvious treatment-related increase in the frequencies of chromosomal aberrations in any of groups receiving test material. NOEL > 70 ppm. UNACCEPTABLE and not upgradeable because the HDT is at least half of a MTD. (Kishiyama, 1/30/89; Rinkus, 4/4/89).

103 066719 "Dominant Lethal Testing in Male Rats," (Inveresk Research International Ltd., Scotland; report no. 1190, 5/30/81). Methyl bromide was administered by whole body inhalation at concentrations of 0 (air), 20 and 70 ppm to 10 male Sprague Dawley rats/treatment level for 7 h/day for 5 consecutive days. After the fifth exposure, males were housed with pairs of virgin, non-treated females for 7 days, with a different pair of females being used weekly for a total of 10 consecutive weeks. Examination of the ovaries and the uterine contents indicated no genotoxic effects or reproductive effects, as can be measured in this assay. NOEL > 70 ppm. UNACCEPTABLE and not upgradeable because the number of males per treatment level was only 10 and the HDT was at least half of a MTD. (Kishiyama, 2/1/89; Rinkus, 4/4/89).

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123-136 099090 "Micronucleus Cytogenetic Assay in Mice" (Putman, D.L. & Morris, M.J.; Microbiological Associates, Inc.; study number T9413.122; 5/17/91). Methyl bromide (purity not stated) was tested for the induction of micronuclei in bone-marrow polychromatic erythrocytes of ICR mice of both sexes. Testing involved one-time intraperitoneal injections of 5 mice/sex/dose and sacrificing them 24, 48 or 72 hours later. Doses based on analytical determinations were: 0 (corn oil), 28, 57, and 123 mg/kg; the targeted low, mid and high doses had been 34, 68, and 136 mg/kg, respectively. The selection of the high dose was based on LD50 data that were contained in the report. **No induction of micronuclei was observed whereas the negative control and positive control (triethylenemelamine, 0.25 mg/kg IP) gave appropriate results. This study is considered **ACCEPTABLE.** (Rinkus, 1/14/92).

123-108 085429 Proposed protocol for conducting a micronucleus test in mice, using intraperitoneal injection as the route of exposure. No worksheet. (Rinkus, 4/20/90).

****123-146 116243** "Toxicology and Carcinogenesis Studies of Methyl Bromide in B6C3F1 Mice (Inhalation Studies)--Micronucleated Peripheral Red Blood Cells," (National Toxicology Program Technical Report 385; March, 1992). Testing was performed at the Brookhaven National Laboratories in New York; the testing was done in two parts using whole-body inhalation. In the initial testing, methyl bromide was administered at concentrations of 0 (air), 12, 25, 50, 100 and 200 ppm for 6 h/d, 5 d/week for a total of 10 exposure days to 5 B6C3F1 mice per sex per treatment level. In subsequent testing, the treatment levels were 0 (air), 10, 20, 40, 80 and 120 ppm for 6 h/d, 5 d/week for 12 weeks, using 8 mice per sex per treatment level. Peripheral blood was collected at the end of exposures in the initial testing and at 4, 8 and 12 weeks during the 12-week studies. Smears were made and processed in a standard manner using acridine orange for staining; and the frequencies of polychromatic and normochromatic red blood cells (RBCs) with micronuclei were determined. In the initial testing, female mice exposed to 100 and 200 ppm exhibited mean frequencies of 9.0 and 16.0 micronucleated RBCs per 1000 cells scored, respectively; these were in comparison to mean frequencies of 3.0-7.0 per 1000 cells scored for the other treatment groups, including the negative controls. **NOAEL = 50 ppm for an 10-day exposure period.** In the 12-week study, no increase in the frequency of micronucleated RBCs was observed for either sex at any of the sampling times. **NOAEL > 120 ppm for exposure periods of 4-12 weeks.** While it may be unexpected that a response would only be seen in the initial testing, without replicate testing or other supplemental information, there presently is no substantial reason to discount this effect. **ACCEPTABLE.** (Rinkus, 1/19/93).

****123-146 116243** "Toxicology and Carcinogenesis Studies of Methyl Bromide in B6C3F1 Mice (Inhalation Studies)--Sister Chromatid Exchanges Testing," (National Toxicology Program Technical Report 385; March, 1992). Testing was performed at the Brookhaven National Laboratories in New York; testing was done in two parts, using in both cases whole-body inhalation and four B6C3F1 mice per sex per treatment level. In the initial testing, methyl bromide was administered at concentrations of 0 (air), 12, 25, 50, 100 and 200 ppm for 6 h/d, 5 d/week for a total of 10 exposure days. In subsequent testing, the treatment levels were 0 (air), 10, 20, 40, 80 and 120 ppm for 6 h/d, 5 d/week for 12 weeks. Twenty-four hours before being sacrificed, the mice received a tablet of bromodeoxyuridine as an implant under their skin; and two hours before being sacrificed, they received an IP injection of colchicine. Bone marrow cells were isolated from the femurs and processed in a standard manner for examination of metaphase spreads for sister-chromatid exchanges (SCEs). Twenty-five second division metaphase cells were scored per mouse. In the initial testing, female mice exposed to 100 and 200 ppm exhibited mean frequencies of 4.8 and 5.3 SCEs/cell, respectively; these were in comparison to mean frequencies of 3.2-3.8 SCEs/cell in the other treatment groups, including the negative controls. **NOAEL = 50 ppm for an 10-day exposure period.** In the 12-week study, no increase in the frequency of SCEs/cell was observed for

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either sex. **NOAEL > 120 ppm for a subchronic exposure.** While it may be unexpected that a response would only be seen in the initial testing, without replicate testing or other supplemental information, there presently is no substantial reason to discount this effect. **ACCEPTABLE.** (Rinkus, 1/19/93).

DNA DAMAGE

Note: EPA is requiring an unscheduled DNA synthesis test using rat hepatocytes and a test to determine the effects on germ cells (see EPA Re-registration Guidance document of Aug., 1986). Presumably, record 162362 was done to satisfy the latter. (Rinkus, 3/5/99).

****044 913095** "In vitro Microbiological Mitotic Recombination Assay of Methyl Bromide Using S. cerevisiae D3." (SRI International, 4-80) Methyl bromide, purity not stated, was assayed for mitotic recombination with Saccharomyces cerevisiae D3 at 0, 0.05, 0.075, 0.1, 0.15, 0.2, 0.3, or 0.4 % w/v. The study was conducted on 4 days, total of 5, 10 or 15 plates per concentration, with and without activation. Increase in number of mitotic recombinants with increasing dose. **Acceptable.** J. Wong, 4-8-85.

103 066718 "Unscheduled DNA Synthesis Assay," (Inveresk Research International Ltd., Scotland; report no. 1190, 5/30/81). Unscheduled DNA synthesis was measured in human embryonic intestinal cell after exposure to methyl bromide gas in air at concentrations of 5, 10, 20, 30, 40, 50, 60, or 70%. None of the methyl bromide treatments induced any increase in UDS. UNACCEPTABLE but upgradeable upon submission of a more detailed explanation of how the cells were exposed to test material, the number of cultures per treatment level, and cytotoxicty data. (Kishiyama, 1/30/89; Rinkus, 4/6/89).

103 066720 "Sperm Abnormalities Test in Mice," (Inveresk Research International Ltd., Scotland; report no. 1190, 5/30/81). Methyl bromide was administered by whole-body inhalation at concentrations of 0 (air), 20, and 70 ppm to 10 B6C3F1 hybrid male mice per treatment level. Mice were sacrificed 5 weeks later and their sperm were catergorized in terms of the frequencies of abnormally shaped sperm. There was no significant increase in the frequency of abnormally shaped sperm in the mice treated with test material. **NOEL > 70 ppm.** UNACCEPTABLE but may be upgraded upon submission of purity of test material and toxicity data that supports the conclusion that 70 ppm is a reasonable approximation of a MTD. (Kishiyama, 2/2/89; Rinkus, 4/5/89).

123-109 087799 "Methylated Purines in Human Liver DNA after Probable Dimethylnitrosamine Poisoning," (Herron, D.C. and Shank, R.C., Cancer Res. 40: 3116-3117, 1980). DNA isolated from the liver and kidneys of a single victim of methyl bromide poisoning (no details at all on this poisoning) did not contain any detectable amounts of 7-methylguanine or O^6-methylguanine. Supplemental information. No worksheet. (Rinkus, 2/22/90).

123-109 087800 "Evaluation of Genetic Risks of Alkylating Agents. IV. Quantitative Determination of Alkylated Amino Acids in Haemoglobin as a Measure of the Dose after Treatment of Mice with Methyl Methanesulfonate," (Segerback et al., Mutation Res. 49: 71-82, 1978). Article does not contain any testing results for methyl bromide per se, but it does explain methods and logic for this approach as applied to methyl bromide in record 087809. Supplemental information. No worksheet. (Rinkus, 2/23/90).

123-108 085428 Proposed protocol for measuring DNA single-strand breakage in the DNA of testicular cells isolated from rats exposed by inhalation. No worksheet. (Rinkus, 4/20/90).

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123-155 129996 This record is a letter dated February 1, 1994 from the Registrant to the Office of Pesticide Programs of USEPA, informing them that DNA damage had been observed using the alkaline elution technique on DNA isolated from testes of male F344 rats. Animals were exposed to 0, 75, 150 or 250 ppm methyl bromide 6 h/d for 5 days, with sacrifice one hour and one day after the 5th exposure. DNA damage was detected with this technique at the high dose at both sacrifice times. Review of tabular data indicates that the effect at 250 ppm was comparable to that produced by the positive control, methyl methanesulfonate at 50 mg/kg (route and total dose not specified). **Supplemental information. No worksheet.** (Rinkus, 7/24/95).

123-188 162362 "Detection of Single Strand Breaks in Rat Testicular DNA by Alkaline Elution Following In Vivo Inhalation Exposure to Methyl Bromide" (K.S. Bentley; Medical Research Project No.: 9714-001 [Sponsor Study No.: MBIP-21.0-ALK-HASK]; March 23, 1994). Methyl bromide (>99% purity) was administered by whole body inhalation for 6 h/day for 5 days to 5 Fischer 344 males/treatment level/sacrifice time. Rats were sacrificed 1 hour or 1 day after the 5th exposure and testicular cells were isolated and analyzed for single-strand DNA breakage using the alkaline elution procedure and a fluorometric assay for DNA determination. Nominal dose levels were: 0, 75, 150 and 250 ppm; the corresponding analytical values were: 0, 77, 153, and 258 ppm. The high dose was selected based on record 087805 (Fund. Appl. Toxicol. 9:352-365, 1987). Also, as controls for the alkaline-elution assay, testicular DNA was processed from 5 males/control/trial that had been injected ip with phosphate-buffered saline or 50 mg/kg methyl methanesulfonate (MMS) and sacrificed two hours posttreatment; two trials were necessitated by equipment failure in the initial trial. The first exposure day resulted in a loss in mean bodyweight for the 150 and 250 ppm groups. On the day after the 5th exposure, the mean bodyweights of the 250 ppm and 150 ppm groups were 78% and 97%, respectively, of what they had been before the onset of exposures; the 0 and 75 ppm groups showed little or no gain in bodyweight over this interval. Two rats from the 250 ppm group died before scheduled sacrifice (test days 5 & 6) and a third rat from this group was sacrificed ahead of schedule (test day 5) due to its moribund state. Signs of neurotoxicity seen in the 250 ppm included ataxia, spasms, diarrhea, lethargy and prostration. Colored nasal discharge was seen in all treatment groups involved in inhalation exposures, including a 40% incidence in the 0 ppm group prior to the final exposure period (not explained). The alkaline-elution curves indicated that the DNA from the 250 ppm group (both sacrifice times) eluted significantly faster than the DNA from the 0 ppm groups and at a rate comparable to that seen with the DNA from the MMS-treated males. The alkaline-elution curves also indicated that the DNA elution rate for the 150 ppm group (1-h posttreatment sacrifice) was significantly slower than the rate seen with the corresponding 0 ppm group (both sacrifice times) and that the amount of DNA retained on the filters at the end of the 15 h of elution for the 75 ppm group (24-h posttreatment sacrifice) was significantly less than that seen with the corresponding 0 ppm group. **LOEL = 75 ppm** (this is a conservative call based on the statistically significant findings reported for the rats sacrificed 24 h posttreatment). This study is considered **UNACCEPTABLE.** Upgrading will require the submission of the following: protocol and raw data for the study; historical control data (negative and positive) from the conducting laboratory; explanation of the time frames per group for inhalation exposures, sacrifices, and alkaline elution runs; slope recalculations with statistical analysis using the combined data for the four negative-control groups. The supplemental information that is being sought is for the purposes of setting the NOEL. Although record 162362 presently is unacceptable, it is sufficient for concluding that inhalation exposure to methyl bromide resulted in DNA damage in rat male germ cells. This is true even after taking into consideration the Registrant's waiver petition to the USEPA (contained in document 123-186) regarding extra testing that was being required based on the results of this alkaline-elution study (discussed in worksheet W162362.844). **Supplemental information.** (Rinkus, 12/14/98).

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NEUROTOXICITY

Note: The brain is clearly a target organ for inhaled methyl bromide (e.g., reviewed in records 059183 & 064742). Comparison of the results of inhalation studies conducted with dogs (records 132821 & 132818), rabbits (records 026865/026866, 095930 & 111266; Irish et al., J. Industr. Hyg. Toxicol. 22:218-230, 1940; Anger et al., Scand. J. Work Environ. Health, 7 [Suppl. 4]: 40-47, 1981; and Russo et al., J. Toxicol. Environ. Health, 14:247-255, 1984), monkeys (Irish et al., J. Industr. Hyg. Toxicol. 22: 218-230, 1940), rats (records 026866/026865, 059184, 087805, 131609 & 131619; Irish et al., J. Industr. Hyg. Toxicol. 22:218-230, 1940; and Anger et al., Scand. J. Work Environ. Health, 7 [Suppl. 4]:40-47, 1981), and mice (record 116243) indicates that there is a significant species difference in sensitivity to the neurotoxic effects of inhaled methyl bromide, with nonrodents (dogs, rabbits, monkeys) being more sensitive than rodents. (Rinkus, 7/24/95).

123-158 131609 "Methyl Bromide: Single Exposure Vapor Inhalation Neurotoxicity Study in Rats" (Driscoll, C.D. & Hurley, J.M.; Bushy Run Research Center; laboratory project ID no. 92N1197; 5/27/93. Methyl bromide (100% purity) was administered to 15 CD® rats/sex/treatment level by whole body inhalation at 0, 30, 100 and 350 ppm, for 6 h. The high dose was selected based on the study by Honma et al. (Tox. Appl. Pharm. 81:183-191, 1985). Neurobehavioral testing utilized 15 rats/sex/group and included automated assessments of motor activity and testing in a functional observation battery. Testing was done: preexposure; within 3 h postexposure; 7 d postexposure; and 14 d postexposure. Rats were sacrificed 16-19 d postexposure. Ten rats/sex/group underwent perfusion fixation. Six rats/sex/group for the 0 ppm and 350 ppm groups had their nervous system and nasal tissue examined histologically. Neurobehavioral effects were only seen in the testing done within 3 h postexposure and only the rats exposed to 350 ppm were affected. Findings included: decreased arousal (both sexes); increased incidence of drooping or half-shut eyelids (both sexes); increased urination (females only); decreased rearing (both sexes); decreased tail pinch response (males only); increased incidence of piloerection (both sexes); decreased rectal temperature (both sexes); abnormal air righting (females only); and decreased motor activity (both sexes). No effects on bodyweight or brain weight were noted. Vacuolation that was seen in the cerebellar white matter and the white matter tracts of the spinal cord for 0 ppm and 350 ppm rats was dismissed as an incidental finding. Otherwise, no histological lesions were noted in the the nervous system or the nasal tissues of the 350 ppm rats. **NOAEL = 100 ppm. Supplemental Information.** (Rinkus, 1/3/95).

123-159 131619 "Methyl Bromide: Ninety-Day Vapor Inhalation Neurotoxicity Study in CD® Rats" (Norris, J.C., Driscoll, C.D. & Hurley, J.M.; Bushy Run Research Center; laboratory project ID no. 92N1172; 9/29/93 [1/5/94 for amendment 1]). Methyl bromide (100% purity) was administered to 15 CD® rats per sex per treatment level by whole body inhalation at 0, 30, 70 and 140 ppm, for 6 h/d, 5 d/week, for 13 weeks. Treatment levels were selected on the basis of subchronic studies conducted previously by the NTP (contained in record 116243). Neurobehavioral testing was done preexposure and at the end of study weeks 4, 8 and 13. Testing included automated assessments of motor activity (13-15 rats/sex/group) and testing in a Functional Observation Battery (9-10 rats/sex/group). Rats used in the FOB testing underwent perfusion fixation. Six rats/sex/group had their nervous system examined histologically in two phases: first phase, 0 and 140 ppm groups; second phase, 30 and 70 ppm groups (amendment 1 [record 131621]). Two 140 ppm males died on test (study days 12 and 27); the latter had convulsions and tremors before dying. One other 140 ppm male that survived till the end of the study also exhibited clonic convulsions and tremors. The 140 ppm groups (both sexes) weighed significantly less than the controls, starting study week ~4; and the 70 ppm female group exhibited a bodyweight reduction, starting study week ~9. FOB testing identified effects only in the 140 ppm groups; some effects were evident as early as study week 4. Findings included: ataxia (five females, one male); decreased arousal (females only); decreased rearing activity (females only); increased hind leg splay (males only); and (possibly) abnormal air righting (males only). Motor activity testing identified effects only in the 70 ppm and 140 ppm female groups. Findings included decreased total motor activity and decreased rearing activ-

MR. 3/5/99

ity; both were evident as incipient effects in study week 8. Female groups exposed to methyl bromide exhibited a dose response for reduced brain weight whereas only the 140 ppm male group had reduced brain weight. Histological findings included: brain lesions at multiple sites (140 ppm, four males affected: neuronal loss, neuronal necrosis, malacia); peripheral nerve degeneration and/or vacuolation (140 ppm, two/sex affected; 30 ppm, one female affected); and olfactory epithelium dysplasia (140 ppm, three/sex affected). White matter vacuolation was seen in the second-phase examination of all males and some females and was considered by the authors to be a storage/pressure artifact. **NOAEL < 30 ppm (reduced brain weight at the lowest dose tested).** When first reviewed (Rinkus, 12/30/94), this study was considered unacceptable and upgrading would require the submission of positive control data. Subsequently, the Registrant submitted records 143173, 143175, 143176 and 143178. These contain results for neurotoxicity testing done by the conducting laboratory using amphetamine, chlorpromazine, acrylamide and (or) iminodipropionitrile. These data did not suffice as positive-control data primarily because the data were too old to be considered contemporary data; also, there were inconsistencies in some of the submissions (discussed in worksheet W131619.S01). Subsequently, the Registrant submitted record 161564 (draft report), which was the "validation" training for the two FOB observers from records 131619 and 131609. These data do not suffice as positive control data, for the reasons discussed in worksheet W131619.S02. Therefore, record 131619 remains **UNACCEPTABLE.** Also, because records 131619, 143173, 143175, 143176, 143178 and 161564 collectively indicate that it is unlikely that adequate positive control data exist to support this study, record 131619 is now considered **NOT UPGRADEABLE. Supplemental Information.** (Rinkus, 1/7/99).

123-160 131621 "Methyl Bromide: Ninety-Day Vapor Inhalation Neurotoxicity Study in CD® Rats: Amendment 1" (Norris, J.C., Driscoll, C.D. & Hurley, J.M.; Bushy Run Research Center; laboratory project ID no. 92N1172 amendment 1; 1/5/94). The histological examination of the rats in record 131619 was done and reported in two phases. Initially, the control and high-dose groups (both sexes) were examined and the results were reported in record 131619. In the second phase, the low and mid-dose groups (both sexes) were examined and the results, combined with the data from record 131619, were reported in record 131621. In this second phase of examinations, vacuolation was noted in the white matter at several sites (e.g., cerebellum, brain stem, trigeminal tract). The authors of amendment 1 dismissed the vacuolation as a "pressure artifact" which developed during storage. This amendment is discussed in worksheet W131619.827. **Supplemental information. No worksheet.** (Rinkus, 7/24/95).

123-170 143173 "Single-Dose Functional Observational Battery Validation Study with Chlorpromazine (CPZ) and Amphetamine (AMP) in Rats" (M.W. Gill; Bushy Run Research Center; BRRC Developmental Project Report 51-902; May 9, 1989). The testing was done with female Sprague-Dawley rats. This record, along with records 143175, 143176 and 143178, were submitted to satisfy the requirement for positive-control data for record 131619 (discussed in worksheet W131619.827). These records are reviewed in worksheet W131619.S01. **Supplemental information.** (Rinkus, 10/15/97).

123-170 143175 "Two-Week Repeated Dose Limb Grip Strength Validation Study with Acrylamide (ACR) in Rats" (Gill, M.W. and Boylstein, L.A.; Bushy Run Research Center; BRRC Developmental Project Report 51-905; Sept. 18, 1989). The testing was done with female Sprague-Dawley rats. This record, along with records 143173, 143176 and 143178, were submitted to satisfy the requirement for positive-control data for record 131619 (discussed in worksheet W131619.827). These records are reviewed in worksheet W131619.S01. **Supplemental information.** (Rinkus, 10/15/97).

123-171 143176 "Single-Dose Motor Activity Validation Study with Chlorpromazine (CPZ) and Amphetamine (AMP) in Rats" (M.W. Gill; Bushy Run Research Center; BRRC Developmental Project Report 51-904; Sept. 18, 1989). The testing was done with F344 rats (both sexes). This record, along with records 143173, 143175 and 143178, were submitted to satisfy the requirement

for positive-control data for record 131619 (discussed in worksheet W131619.827). These records are reviewed in worksheet W131619.S01. **Supplemental information.** (Rinkus, 10/15/97).

123-171 143178 "Two-Week Repeated Dose Functional Observation Battery Validation Study with Acrylamide (ACR) and Iminodipropionitrile (IDP) in Rats" (M.W. Gill; Bushy Run Research Center; BRRC Developmental Project Report 51-903 Revised; Sept. 18, 1989). The testing was done with female Sprague-Dawley rats. This record, along with records 143173, 143175 and 143176, were submitted to satisfy the requirement for positive-control data for record 131619 (discussed in worksheet W131619.827). These records are reviewed in worksheet W131619.S01. **Supplemental information.** (Rinkus, 10/15/97).

123-205 161564 This was a training exercise conducted in November, 1992. One part was intended to train 7 people to perform a functional observation battery (FOB), including the two observers who did the FOB testing in records 131619 and 131609. It was indicated that record 161564 was their "validation" training, qualifying them to do FOB testing in a definitive study. For the FOB "validation" training, 9 male CD rats were used; and d-amphetamine (3 rats; 10-15 mg/kg, ip), carbaryl (2 rats; 19 and 21 mg/kg, ip) and ethanol (2 rats; 7 g/kg, po) served as the neurotoxic standards, with saline (2 rats; po) as the negative control. The other testing in record 161564 exclusively involved the two observers who did the FOB testing in records 131619 and 131609. They measured grip strength (fore and hind) and hind leg splay in untreated male CD rats. Record 161564 was marked "draft" on each page. There were no signatures on the GLP page nor in the "Review and Approval" section; and there was no QA page. **Supplemental information.** (Rinkus, 3/5/99).

SUPPLEMENTAL STUDIES

BRAIN TYROSINE HYDROXYLASE AND BRAIN CATECHOLAMINE STUDIES

No Record Number. "Inhibition of Tyrosine Hydroxylase Activity by Methyl Bromide Exposure" (Honma et al., Neurotoxicology and Teratology 13:1-4, 1991). Male Sprague-Dawley rats (3 to 5 rats/dose level/sacrifice time) were exposed to methyl bromide (0 to 250 ppm) for 8 h using inhalation chambers. The animals were sacrificed 0, 1, 2 or 24 hours postexposure. Brain tyrosine hydroxylase (THase) activity was quantitated in an "in vitro" assay and in an "in vivo" one. Both assays indicated dose-responses for decreases in DOPA production in various brain segments. The segment with the lowest effect level (LEL) in the "in vitro" assay was the hypothalamus; its LEL was 16 ppm, the lowest dose tested. The segments with the lowest effect level (LEL) in the "in vivo" assay were the striatum and hypothalamus; their LEL was 63 ppm, with a possible incipient effect at 31 ppm. The maximal inhibition of THase activity in both assays was seen with the rats sacrificed immediately after the 8 h exposure period; significant recovery took place within two hours postexposure and was complete by 24 h postexposure. The authors interpreted their findings as evidence that methyl bromide directly caused changes in the enzyme structure, presumably by methylation. However, as reviewed in worksheet "whonma1.sup," there are significant questions about the findings of Honma et al. (1991) and its relationship to other studies. **Supplemental information.** (Rinkus, 1/26/98).

No Record Number. "Significant Changes in Monoamines in Rat Brain Induced by Exposure to Methyl Bromide" (Honma et al., Neurobehavioral Toxicology and Teratology 4:521-524, 1982). In one part of this study, male Sprague-Dawley rats were exposed for 24 h to 0, 10, 20, 40, 60, 100 or 120 ppm methyl bromide. In another part, rats were exposed for 3 weeks to 0, 1, 5 or 10 ppm methyl bromide. In both parts, rats were sacrificed immediately after exposure using a focussed microwave pulse directed at the head. The brain was sectioned into segments and several neuro-

transmitters (norepinephrine, dopamine, serotonin, acetylcholine) and cyclic nucleotides (cAMP and cGMP) were assayed. The main finding was that significant reductions in norepinephrine occurred in the hypothalamus and in a segment consisting of the cortex plus hippocampus. The reductions were seen in the groups exposed to 100 and 120 ppm for 24 h and in the group exposed to 10 ppm for 3 weeks. Although norepinephrine was reduced, dopamine in the striatum was unchanged or possibly increased. The lack of a reduction in dopamine is inconsistent with the main premise of Honma et al. (1991), which is that methyl bromide affects THase, causing a decrease in DOPA, which in turn leads to decreases in dopamine and norepinephrine. **Supplemental information. No worksheet.** (Rinkus, 4/15/98).

No Record Number. "Methyl Bromide Alters Catecholamine and Metabolite Concentrations in Rat Brain" (Honma et al., Neurotoxicology and Teratology 9:369-375, 1987). In the first part of this study, male Sprague-Dawley rats were exposed for 8 h to 0 or 100 ppm methyl bromide and sacrificed at 0, 2, 8 or 24 hours postexposure. In the second part, rats were exposed for 8 h to 0, 31, 63, 125 and 250 ppm methyl bromide and sacrificed immediately afterwards. A focussed microwave pulse to the head was used to sacrifice the animals. The brain was sectioned into the same segments used in Honma et al. (1991). The following neurotransmitters and their respective metabolites were assayed: dopamine and homovanillic acid; norepinephrine and 3-methoxy-4-hydroxyphenylglycol (MHPG); and serotonin and 5-hydroxyindoleacetic acid (5HIAA). The findings from the study were: a) dopamine was decreased (LEL = 100 ppm [striatum]) whereas homovanillic acid was increased (LEL = 63 ppm [striatum, hypothalamus]); b) norepinephrine was decreased (LEL = 31 ppm [hypothalamus]) whereas MHPG was increased (LEL = 63 ppm [striatum, hypothalamus, midbrain]); c) serotonin and 5HIAA were not significantly affected in any brain segment (LEL > 250 ppm); and d) return of dopamine, homovanillic acid, norepinephrine and MHPG to their respective control values was complete by 24 h postexposure. Some inconsistencies that this study presents include the following. First, decreased dopamine was measured in the striatum of rats exposed for 8 h to 100 or 125 ppm methyl bromide whereas exposure at these same levels for a much longer period, 24 h, did not affect dopamine content in Honma et al. (1982). Second, if THase is inhibited as proposed in Honma et al. (1991), one would expect that the metabolites "downstream" from THase would be decreased. That is, the DOPA decrease should lead to a decrease in dopamine, which in turn should cause a decrease in the dopamine catabolite, homovanillic acid. This is what occurs when α-methyl tyrosine (methyl ester), a known THase inhibitor, is given to rats (Aust. J. Biol. Sci. 36:519-523, 1983). However, the opposite occurred in this study: homovanillic acid increased, with a LEL (63 ppm) that was lower than the LEL for the dopamine decrease (100 ppm). **Supplemental information. No worksheet.** (Rinkus, 4/15/98).

No Record Number. "Behavioral Evidence for Modified Receptor Sensitivity in Rat Brain Induced by Methyl Bromide Exposure" (Honma et al., Industrial Health 32:1-16, 1994). This study was a follow-up to the work reported in Honma et al. (1991). The intent was to test whether male Sprague-Dawley rats exposed to methyl bromide were more sensitive (responsive) to the dopamine agonist apomorphine, which causes hyperactivity in rats. Increased sensitivity to a dopamine agonist was expected if methyl bromide had damaged presynaptic neurons that use dopamine as the neurotransmitter. The increased sensitivity was thought to be a way to compensate for the presynaptic damage, by having the postsynaptic neurons increase their density of dopamine receptors and (or) increase the affinity of their receptors for dopamine. Also, whether methyl bromide affected the hypoactivity induced by the norepinephrine agonist clonidine was tested. Two assays were used. One assay involved a blind scoring of the stereotypic oral behavior (defined as abnormal sniffing, licking and biting) caused by an i.p. injection of apomorphine. This assay used five rats/dose level and was conducted 7 days before exposure to methyl bromide and on days 1, 4, 7, 14 and 28 postexposure. There were two types of inhalation exposure: 8 h to 0, 25, 50, 100 or 200 ppm; and 8 h/day for 7 consecutive days to 0, 5, 10, 25 or 50 ppm.The second assay involved measuring locomotor activity in an automated-counting apparatus after an i.p. injection of apomorphine or clonidine. This assay used only two rats/dose level and was conducted 7 days after exposure to 0, 10 or 50 ppm methyl bromide (8 h/day for one day or for 7 consecutive days). Testing also was done the day before exposure to methyl bromide; in these instances, neither apomorphine

MR 2/5/99

4/15/99

nor clonidine were administered before the locomotor activity was recorded. The DPR MT reviewer's concerns about this study are contained in the review of Honma et al. (1991) (i.e., worksheet "whonma1.sup"). **Supplemental information. No worksheet.** (Rinkus, 4/15/98).

SINGLE AND (OR) REPEATED INHALATION EXPOSURE STUDIES

No Record Number. "The Response Attending Exposure of Laboratory Animals to Vapors of Methyl Bromide" (Irish et al., J. Ind. Hyg. Tox., 22:218-230, 1940). This study involved single exposures of rats and rabbits and repeated exposures for up to 6 months (7.5-8 h/d, 5 d/w) to rats, guinea pigs, rabbits, and rhesus monkeys (rodent and rabbit strains not specified). The study is notable for its findings of neurotoxicity and species differences. The results suggest the following decreasing order of sensitivity to the neurotoxic effects of repeated exposure to methyl bromide-containing atmospheres: rabbits ≥ monkeys > guinea pigs ≥ rats. Literature reference. (Rinkus, 1/17/92).

4-17 WEEK GAVAGE STUDY, MALE RATS

083 059183 "The Subchronic Effects of Oral Methyl Bromide Administration in the Rat," (Purdue University, Masters Thesis, Ann Frances Hubbs, December, 1986). Methyl bromide was administered by gavage at the nominal concentrations of 0 (peanut oil), 25, and 50 mg/kg/day (5 days/week) to 71, 41, and 71 male Wistar rats, respectively. Rats received treatments until sacrificed at 4, 9, 13, or 17 weeks, with 7-10/group/sacrifice; however, rats in the 25 mg/kg/day group were not sacrificed at the two earliest times. Also, some rats in each group stopped receiving treatments after 13 weeks and remained untreated for either 4 or 9 weeks before being sacrificed. Toxicological examination mainly consisted of histological examination of blood, bone marrow and stomach. Food consumption and bodyweights were reduced in both groups receiving methyl bromide. Gross and histological changes were observed in the stomach of most rats receiving methyl bromide and were consistent with damage and inflammation of the squamous epithelium, but no tumorigenesis was indicated. NOEL, MTD < 25 mg/kg/day. Supplemental information. (Kishiyama, 1/24/89; Rinkus, 4/17/89).

Note: record 059183, as a thesis, contains an extensive literature review on methyl bromide. Topics include: poisoning in man by dermal, ocular (?), inhalation, and oral exposure; experimental animal studies; and in vitro studies (mutagenicity, transformation, and cytotoxicity). (Rinkus, 4/25/89).

TOXICOLOGY LITERATURE REVIEW

099 64742 "Toxicology of Methyl Bromide" is some sort of collaborated review, 29 pages long, plus 7 pages of references (with first two pages missing). Authors have affiliations with Toxicology and Pharmacology, Inc., Georgetown University, and Virginia Commonwealth University Medical College of Virginia. The authors' purpose in preparing the review (e.g., as a submission for publication) is not indicated; also, there is no date on the manuscript. Topics include: exposure, pharmacokinetics, human health effects, experimental studies, teratogenic activity, mutagenic activity, carcinogenic activity, and mechanism of action. It was noted that the most recognized effect of methyl bromide was neurotoxicity. No worksheet. Supplemental information. (Rinkus, 4/25/89).

SJR 3/5/99

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RESIDUE STUDIES

Note: Record 126281 demonstrated that methyl bromide is readily converted to methyl chloride in the presence of water and sodium chloride. Given the facile production of methyl chloride when animal feed is fumigated, these data indicate that methyl chloride may be a concomitant residue after methyl bromide fumigation of organic matricies containing water and chloride (e.g., feeds and foods). (Rinkus, 7/24/95).

123-109 087810 "Methyl Bromide Residue Study (Pre-Plant)--Revised Draft," (Bolsa Research Associates; B.R. #10:87, 4/11/88). This record is some sort of partial report on results of measuring organic methyl bromide and inorganic bromide in a variety of crops grown on soil fumigated with methyl bromide. Apparently, no methyl bromide was detected in any crops grown on fumigated soil, while inorganic bromide levels were increased. Supplemental information. Not reviewed; no worksheet. (Rinkus, 4/20/90).

123-109 087811 "Section E: Removal of Residues," (no author or other identification given). This record is some sort of partial report regarding "additional means of reducing methyl bromide residues," presumably after commercial fumigation. Supplemental information. No Worksheet. (Rinkus, 4/20/90).

123-109 087812 "Fumigant Survey: Flour and Flour Products, April-June 1984," (Oregon Department of Agriculture, Laboratory Services Division, Food and Dairy Division; no date). No methyl bromide was detected in 100 flour and bakery mix products. The analytical method that was used had a detection limit of 0.03 ppm. Supplemental information. No worksheet. (Rinkus, 4/20/90).

123-109 087813 "Determination of Methyl Bromide Residues in Strawberries after Commercial Fumigation," (no author or other identification given). This record is some sort of partial report regarding the loss of organic methyl bromide residues from strawberries fumigated at the Driscoll Strawberries Associates fumigation facility in Watsonville, CA. The analytical method that was used was the headspace gas-chromatography assay of King et al. Data which were not provided were said to indicate an exponential loss in organic residues, such that only 3×10^{-6} ppm would be expected after 8 hours of some sort of unspecified aeration. Supplemental information. No worksheet. (Rinkus, 4/20/90).

123-151 124366 This record is a letter dated June 8, 1993 from the Registrant to the Office of Pesticide Programs at USEPA. An accompanying letter (no record number) in the front of 123-151 (dated June 16, 1993 and addressed to Dr. Larry Nelson [DPR MT Branch Chief]) indicates that the ecotoxicity testing data in record 124366 on the stability of methyl bromide in water is relevant to the discussion of how to conduct the rat chronic feeding study. Although it is stated that the rapid loss of methyl bromide from water makes it unacceptable to perform a chronic toxicity study using drinking water, no data concerning the losses incurred using dinking-water bottles were provided. Tabular data indicate that 10 mg/L solutions of methyl bromide in "well water" contained 86-89% of their initial content 48 h later (experimental conditions not described--apparently a closed system). **Supplemental information. No worksheet.** (Rinkus, 7/24/95).

123-154 126281 "Study to Determine the Feasibility of Preparing Dog and Rodent Diet with a Controlled Methyl Bromide Residual" (Ariano, J.; Great Lakes Chemical Corp.; technical report number: 1-93-10; Aug. 21, 1993). This record is notable for the following: its analytical studies utilized a modification of the headspace assay of King et al. (J. Agric. Food Chem. 29:1003-1005, 1981); it documented that in the fumigation of animal feed, there is sufficient water and chloride content to result in the formation of methyl chloride, presumably through some halide exchange reaction. DPR MT's concerns about the modified assay of King et al. are discussed in the rebuttal response of July 24, 1995 (R950724). **Supplemental information. No worksheet.** (Rinkus, 7/24/95).

SOR 3/5/99 232 3/5/99

123-151 124373 This record is a "working draft" (dated 5/9/93) of record 126281 (dated 8/21/93). This record has not been reviewed since it was superseded by record 126281. **Supplemental information. No worksheet.** (Rinkus, 7/24/95).

MISCELLANEOUS

123-140 112312 This record contains a letter (dated 1/2/92) from Dee Kuhn (the Chemical Manufacturers Association Manager for the MBIP). The letter summarizes a variety of matters discussed at the meeting of October 30, 1991 in Sacramento between the representatives of the MBIP and CDPR MT staff. This record also contains some written text and tables regarding the presentation made on methyl bromide neurotoxicology at the aforementioned meeting by Dr. Michael Gill. **Supplemental information. No worksheet.** (Rinkus, 1/17/92).

NOTE: All studies received by the DPR Medical Toxicology Branch up to 4/15/98 have been considered in this SUMMARY OF TOXICOLOGY DATA. The following also have been received but have not been reviewed:

1) 123-172 143942 "A Four Week Dietary Range-Finding Toxicity Study of Methyl Bromide in Rats" (E. Crosby Tompkins; project number WIL-49013; Aug. 16, 1995 [Susan Lewis's signature]).

2) 123-173 143943 This is a copy of a memorandum from from Dr. Vince Piccirillo (NPC, Inc.) to Dr. Sue Lewis (CMA/MBIP) dated Nov. 27, 1995. The subject matter is identified as: "Six-Month Status for WIL Research Laboratories Study No. WIL-49014: A 24-Month Chronic Dietary Toxicity Study of Methyl Bromide in Rats."

3) 123-176 146039 This is an interim letter report regarding the one-year status of project number WIL-49014 (A 24-Month Chronic Dietary Toxicity Study of Methyl Bromide in Rats). The report is from J.J.W.M. Mertens (Study Director, WIL) to Susan A. Lewis (Methyl Bromide Industry Panel/CMA). It is dated March 26, 1996. Presumably, it is a synopsis of record 149113.

4) 123-177 149113 This is a 1767-page presentation of the data through test week 52 for project number WIL-49014 (A 24-Month Chronic Dietary Toxicity Study of Methyl Bromide in Rats). It is dated August 15, 1996 The author is J.J.W.M. Mertens (Study Director, WIL).

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3/5/99