

METHYL BROMIDE

**RISK CHARACTERIZATION DOCUMENT
INHALATION EXPOSURE**

Addendum to Volume I

Medical Toxicology Branch
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I. INTRODUCTION

The Department of Pesticide Regulation (DPR) has conducted risk assessments to address the potential risk associated with human exposure to methyl bromide in California. In 1999, a draft Risk Characterization Document (RCD) for inhalation exposure (DPR, 1999) was reviewed by a Subcommittee of the National Research Council (NRC, 2000). The Subcommittee recommended additional toxicology studies, in particular a new subchronic toxicity study, and exposure monitoring to better characterize the risk. In 2002, DPR finalized the RCD for this exposure route (referred to as the 2002 RCD in this document) with major changes in the exposure assessment (DPR, 2002a). DPR also finalized the risk characterization documents for dietary and aggregate exposures (DPR, 2002b and 2002c).

Subsequent to the completion of the 2002 RCD, the registrant submitted a new subchronic toxicity study in dogs (Schaefer, 2002) in response to the NRC comments. Since the choice of the appropriate subchronic endpoint and No-Observed-Effect Level (NOEL) is critical to both the risk assessment and risk mitigation measures, DPR conducted a series of scientific reviews to determine if the critical NOEL for subchronic exposure used in the 2002 RCD needed to be revised. The initial reviews focused on the determination of the NOEL for the Schaefer study. They were conducted by the Medical Toxicology staff (Appendix A; Kellner, 2002 in Appendix B; Patterson, 2002 in Appendix D) and by Dr. Pinkerton, University of California at Davis (Pinkerton, 2002 in Appendix C). While there were varied scientific interpretation of the data, the general consensus was that the NOEL for the Schaefer study was 5 ppm instead of the 20 ppm proposed by the study author and the registrant's reviewer, Dr. J. Chambers (Schaefer, 2002; Chamber, 2002). After these reviews, DPR reevaluated the subchronic toxicity database taking into consideration these review comments and proposed a revision of the critical subchronic NOEL (Appendix E). This proposal and relevant documents were submitted to a formal external scientific review¹ by Dr. J. Last, University of California at Davis. In his review, Dr. Last recommended the use of 5 ppm as the NOEL for subchronic exposure and his rationale is provided in Appendix F. DPR concurred with the recommendation and concluded that the critical NOEL should be revised to 5 ppm (Schaefer, 2002), compared to the estimated NOEL of 0.5 ppm used in the 2002 RCD. This Addendum updates the 2002 RCD with the revised margins of exposure for subchronic exposure; the margins of exposure for other durations of exposure are not affected by this revision. Since the subchronic exposure scenario was not considered in the risk characterization documents for dietary and aggregate exposures, this change in the NOEL does not have any impact to the conclusions of those documents.

¹The review was conducted under a Task Order (TO 38-1), as pursuant to the provisions of Interagency Master Agreement between the California Environmental Protection Agency and the Regents of the University of California, Davis, for scientific peer review. Documents submitted for the review included: Selection of Subchronic Critical NOEL for Methyl Bromide Subchronic Inhalation Toxicity (Appendix E), Methyl bromide risk characterization document (DPR, 2002), National Research Council review (NRC, 2002), the dog studies (Newton, 1994a and Schaefer, 2002), DPR's reviews of the two dog studies, Memorandum from Dr. Kellner (Kellner, 2002), and Letter from Dr. Pinkerton (Pinkerton, 2002).

II. HAZARD IDENTIFICATION

For subchronic inhalation exposure, the critical toxicological endpoint was neurotoxicity, which included neurobehavioral changes and effects in the brain (reduced brain weight and lesions). Studies pertinent for the selection of critical NOEL are the dog studies (Newton, 1994a and b; Schaefer, 2002), and three rat studies (Eustis *et al.*, 1988; NTP, 1992 and Eustis, 1992; and Norris *et al.*, 1993 a and b). A detailed description of these studies and issues relating to the interpretation of the results are in Appendix E.

The possible NOELs from the selected studies are provided in Table 1 (same as Table 7 of Appendix E, except with the distinction of adult and child human equivalent NOELs and inclusion of more significant figures to the values). All these studies showed NOELs ranging from <5 ppm (Newton, 1994a) to < 30 ppm (Norris *et al.*, 1993 a and b) with the reference concentrations for human exposure ranging from 1 ppb to 35 ppb for children and 2 ppb to 63 ppb for adults. For the Newton study, two estimated NOELs (ENEL) are listed depending on what uncertainty factor is used for the extrapolation of NOEL from the LOEL. In the 2002 RCD, the uncertainty factor was a 10-fold default factor. Since review of the database showed that the actual NOEL is close to the LOEL, a smaller UF such as 3-fold could be justified (Appendix E). For the Schaefer study, three possible NOELs are listed to reflect the varied opinions about the study. The majority of the reviewers considered 5 ppm as the NOEL for the study and this NOEL was selected as the critical NOEL. This critical NOEL and those for other exposure durations used in the 2002 RCD are presented in Table 2.

Table 1. No-Observed-Effect Levels and endpoint for methyl bromide subchronic toxicity.

Studies	Species/ Duration	Effect	NOEL/ LOEL (ppm)	ENEL ^a (ppm)	Human Equivalent NOEL ^b		Reference concentration ^c	
					Adult	Child	Adult	Child
Subchronic Exposure (6-13 weeks)								
Newton, 1994a	Dog/ 6 weeks	Unrespon- siveness	<5 / 5	0.5 UF=10	0.16 ppm	0.09 ppm	2 ppb	1 ppb
				1.7 UF=3	0.53 ppm	0.30 ppm	5 ppb	3 ppb
Schaefer, 2002	Dog/ 6 weeks	Tremors, twitching, emesis	<5/5	1.7 UF=3	0.53 ppm	0.30 ppm	5 ppb	3 ppb
		Absence of Proprio- ceptive placing response	5 / 10	NA	1.56 ppm	0.88 ppm	16 ppb	9 ppb
		No Effects	20/ >20	NA	6.25 ppm	3.53 ppm	63 ppb	36 ppb
Norris <i>et al.</i> , 1993 a and b	Rat/ 13 weeks	Brain weight reduction	<30/30	3 UF=10	1.98 ppm	1.12 ppm	20 ppb	11 ppb

a/ In the absence of a No-Observed-Effect Level (NOEL), the Lowest-Observed-Effect Level (LOEL) is divided by an uncertainty factor (UF) to estimate a NOEL. The default UF may be 3 or 10-fold depending on the severity of the effect.

b/ Human equivalent NOELs take into consideration of respiratory rate differences between experimental animals and humans (based on children rate of 0.46 m³/kg/day and adult respiration rate of 0.26 m³/kg/day) and amortized for 24 hours of exposure. The default respiration rates for dogs and rats are 0.39 m³/kg/day and 0.96 m³/kg/day, respectively. For example, the calculation for adult human equivalent NOEL based on the 5 ppm from the Schaefer study is:

$$5 \text{ ppm} \times \frac{0.39 \text{ m}^3/\text{kg}/\text{day}}{0.26 \text{ m}^3/\text{kg}/\text{day}} \times \frac{7 \text{ hours}}{24 \text{ hours}} \times \frac{5 \text{ days}}{7 \text{ days}} = 1.56 \text{ ppm}$$

c/ Reference concentration is 1/100 of the human equivalent NOEL.

Table 2. Critical No-Observed-Effect Levels and endpoints for methyl bromide risk characterization.

Scenarios	Experimental NOEL	Human Equivalent NOEL ^a		Reference Concentration ^d	Effects in Animal Studies	Ref ^e
		Adult ^b	Child ^c			
Acute	40 ppm	21 ppm	na	210 ppb	Developmental toxicity (pregnant rabbit)	1*
	103 ppm ^f	45 ppm	25 ppm		Neurotoxicity (dog)	2
Subchronic						
1 week	20 ppm	12 ppm	7 ppm	120 ppb (adult) 70 ppb (child)	Neurotoxicity (pregnant rabbit)	3
6 weeks	5 ppm	0.56 ppm	0.88 ppm	16 ppb (adult) 9 ppb (child)	Neurotoxicity (dog)	4
Chronic	0.3 ppm (ENEL)	0.2 ppm	0.1 ppm	2 ppb (adult) 1 ppb (child)	Nasal epithelial hyperplasia/ degeneration (rat)	5*

^{a/} Experimental NOELs were converted to human equivalents using equations in Attachment G. na= child equivalent NOEL were not calculated because the effects were observed in pregnant animals.

^{b/} The adult equivalent NOELs are appropriate to address worker exposures. They are also used for residential exposures when child equivalent NOELs were not calculated.

^{c/} The child equivalent NOELs are appropriate to address resident exposures (see footnote b).

^{d/} The reference concentration is the ratio of the human equivalent NOEL and a default uncertainty factor of 100 since the NOEL was derived from experimental animal studies.

^{e/} * indicates study was acceptable to DPR according to FIFRA guidelines. References: 1. Breslin *et al.*, 1990; 2. Newton, 1994b; 3. Sikov *et al.*, 1981; 4. Schaefer, 2002; 5. Reuzel *et al.*, 1987 and 1991.

^{f/} The NOEL and human equivalents are presented in this Table for comparison purposes only. They are not used for risk characterization.

III. EXPOSURE ASSESSMENT

The exposure estimates for workers and residents are the same as those in the 2002 RCD (Tables 19-22 in DPR 2002a) and the technical summary is provided below.

III. A. Occupational Exposure

The inhalation exposures of applicators in structural fumigation were not determined because they are required to wear self-contained breathing apparatus. No data were available for other workers such as tarp removers.

For field fumigation, monitoring studies were conducted primarily to determine the effectiveness of modifications to existing application procedures and aeration of treated fields. With shallow-shank and tarp fumigation, workers involved in the application with no modifications had higher exposures than those in other methods. The acute exposures of applicator, copilot, and shovel-man ranged from 188 ppb to 245 ppb. The best method involved both swept-back shank and closing shoes where the applicators, copilots, and shovel-men exposures were 1 ppb to 58 ppb. The driver (7 ppb) and copilot (62 ppb) of the tractor in the placement of tarp had lower acute exposures than those involved in the application. For tarp cutting and removal, one study showed acute exposures of 202 ppb and 215 ppb while another study showed workers with higher acute exposures (22 to 1058 ppb). With deep-shank injection, the applicators with only overhead fan had the highest acute exposure at 281 ppb. Lower acute exposures were measured for applicators in tractors with modifications such as overhead fan and scrapers and rollers (104 ppb), enclosed cab (161 ppb and 171 ppb), and enclosed cab with scrapers (13 ppb). When a second tractor with a disc or cultipacker was involved, the drivers had relatively lower exposure (13-181 ppb) than those for applicators, except for the disc driver (934 ppb). For both short-term and subchronic exposures in shallow-shank and deep-shank methods, the exposure patterns were similar to those for acute exposures, which were the basis for the calculations. Chronic exposure was not expected for any of the work scenarios. For workers at adjacent fields, there were no data and their exposures were assumed to be at 210 ppb.

For workers with potting soil in greenhouses, the maximum acute exposure was 210 ppb. Their actual exposures were relatively low because tarp venters are required to wear self-containing breathing apparatus, and tarp removal occurs after 48 hours of venting. The short term exposures, based on measured values, were 0.001 ppb and 0.14 ppb for these two group of workers. No subchronic or chronic exposures were determined for this activity. No data were available for other workers, e.g., applicators, associated with this use.

For commodity fumigation workers, the acute exposure was 210 ppb and the exposures for other durations based on the average of measured values. For workers involved in the fumigation of grain products, the range of short-term exposures was 0.02 ppb to 11 ppb. The forklift drivers of sea containers/trailers had higher subchronic and chronic exposures (8 ppb) than those (3 ppb) for non-certifying fumigation chambers. For workers involved in the fumigation of raisins, the range of short-term exposures was 3 ppb to 180 ppb. For workers in a walnut processing plant, workers in clearing plant (178 ppb) and vacuum chamber (180 ppb) had the highest short-term exposure compared to other areas. The lowest average short-term level

(25 ppb) was measured in the special cracking area. For both raisin and walnut workers, the short-term and subchronic exposure levels were similar. Chronic exposure was considered for raisin processing workers but was not expected for most walnut processing workers.

For workers in a brewery, exposures were estimated for applicators and aerators at various locations. The acute exposure was assumed to be at 210 ppb. The short-term exposure level ranges were 7-49 ppb for aerators and 8-12 ppb for applicators. No seasonal or chronic exposures were expected.

For workers in the facilities but whose tasks were not directly related to commodity fumigation, data were available only for raisin and walnut fumigations. The exposure levels were either based on the acute level of 210 ppb or measured by ambient and area sampling. The range of short-term exposures ranged from 7 ppb to 180 ppb. The subchronic and chronic exposures (except for walnut processing) were comparable to those for short-term levels because of the frequency of exposure.

III. B. Residential Exposures

The exposures of residents returning to homes after fumigation and aeration were not estimated due to lack of data on current practices. DPR regulations limit the maximum acute exposure at 210 ppb.

Residential exposures to field fumigation were determined using monitoring data and computer modeling of the data². Maximum methyl bromide air concentration was related to the size of the field and emission rate (depending on the method of application). At the 95th percentile, the exposure ranges for each field sizes were: 161-174 ppb (1 acre), 163-215 ppb (10 acre), 201-225 ppb (20 acres), 213-230 ppb (30 acres), and 221-236 ppb (40 acres).

The acute exposure for residents living near commodity fumigation facilities was limited to 210 ppb. The exposures for the longer-term durations were 90-180 ppb (short-term), 70-175 ppb (subchronic), and 86-106 ppb (chronic).

For residents living in methyl bromide use areas, which may include field, commodity, and structural fumigations, ambient air monitoring at the 95th percentile daily exposure levels ranged from 0.239 ppb (Mettler Fire Station) to 30.2 ppb (Pajaro Middle School in Watsonville). Levels at these two sites also provided the ranges for weekly (0.163 to 17.1 ppb), and 7-8 week (0.084 to 7.68 ppb) exposure durations. Additional monitoring has been conducted by the Air Resources Board and the registrant to characterize the exposures (these data are not available yet).

² Available field monitoring data were combined with computer modeling to generate the maximum air concentration or distance under a wide variety of conditions due to the field size, flux (emission rate), and meteorological conditions. The cumulative frequency distribution reflected the maximum concentrations or distances under the 24-hour meteorological data sets (7166 days from 20 years of data). It should be noted that the maximum concentration determined in these analyses occurred only on a portion of the buffer zone perimeter, and these analyses addressed only acute exposure.

IV. RISK CHARACTERIZATION

The margins of exposure (MOEs) for subchronic inhalation exposure of workers and residents were calculated using human equivalent NOELs for the critical NOEL of 5 ppm (Table 1). These human equivalent NOELs account for respiratory rate differences between experimental animals and humans and are amortized for daily exposure (footnote b in Table 1). The workers and residents were represented by adult (1.56 ppm) and child (0.88 ppm) equivalent NOELs. While the critical NOEL increased 10-fold from 0.5 ppm to 5 ppm, these equivalent NOELs are 7.8-fold and 8.8-fold higher than those (200 ppb and 100 ppb) used for adults and children, respectively, in the 2002 RCD due to rounding of values. The revised calculated MOEs are presented in bolded print in Tables 3 to 6. The MOEs for other durations of exposure are included for a more complete discussion of the exposures. The exposure values are the same as those in Tables 19-22 in the 2002 RCD.

IV. A. Occupational Exposure

IV. A. 1. Structural Fumigation

There was no subchronic exposure for workers involved in structural fumigation. The acute MOE for the applicators was assumed to be greater than 100 since these workers are required to be in a self-contained breathing apparatus.

IV. A. 2. Field Fumigation

With shallow-shank/tarp fumigation (Table 3a to b), the subchronic MOEs remained less than 100 for almost all applicator and copilot exposures. For this type of application, the acute exposures of the copilots were also less than 100 in two cases (86 for broadcast and 69 for when closing shoes were used). For shallow-shank/tarp/bed fumigation which included drip tape and closing device (Table 3c to d), all MOEs were greater than 100. The MOEs for workers in tarp cutting and removal varied depending on the study even though similar procedures were used. More measurements were available in the first study (3 to 12 samples) compared to the second study (1 sample each). In study 1 (Table 3e), the MOEs for all durations were close to or greater than 100 for both cutters and pullers, except for a subchronic MOE of 87 for the cutter. In the second study (Table 3f), the cutter and remover: end puller MOEs were close to or greater than 100 for all durations. The acute, short-term, and subchronic MOEs for the other removers (tractor driver and basket-man) remained below 100.

With deep-shank injection with overhead fan (Table 3g and h), the applicators with only overhead fan had the lowest MOE of 75 for acute exposure and 33 for subchronic exposure. MOEs were close to or greater than 100 for other workers and exposure durations. For deep-shank injection with enclosed cab (Table 3i), the MOEs for all durations for disc driver were only 22 (acute), 40 (short-term), and 10 (subchronic). The subchronic MOEs for the other workers were also less than 100 (ranged from 54 to 74). The use of enclosed cab and scrapers resulted in MOEs of greater than 100 for all workers (Table 3j).

For workers at adjacent fields, the acute MOE could be assumed to be 100 with the exposure not to exceed 210 ppb.

IV. A. 3. Commodity/Brewery Fumigation

The acute MOEs for all workers in commodity fumigation facility were 100 because their upper exposure limit was 210 ppb (Tables 4 and 5). For tarp ventors and removers of potting soil fumigation in greenhouses, the MOEs for short-term exposures were greater than 80,000 because of their relatively low actual exposures (Table 4a). No data were available for other workers.

In the fumigation of grain products (Table 4b) and brewery (Table 4d), MOEs for these workers were greater than 100 for all exposure periods. For workers involved in the fumigation of dried fruit and tree nut products, the MOEs were greater than 100 only for forklift drivers. For other workers, the MOEs were generally greater than 100 for acute and short-term exposures but less than 100 for subchronic (11 to 78) and chronic (2 to 25) exposures. For workers in a walnut processing plant, the acute MOE was 67 for workers with the highest exposures (in clearing plant or vacuum chamber, Table 4c2). This MOE was based on measured values (cleaning plant) and the 210 ppb limit (vacuum chamber). The highest MOE was 480 for workers at the special cracking area. The MOEs for subchronic and chronic exposures were less than 100.

For workers in fumigation facilities not directly related to fumigation, the short-term exposure MOEs were generally greater than 100 (MOE of 121 to 1714) for raisin facilities (Table 5a). The short-term MOE for walnut processing was 500 based on area sampling but was 67 based on 210 ppb as the daily exposure level in sorting and packaging areas (Table 5b.2). However, the subchronic and chronic exposure MOEs for both raisins and walnut processing facilities were generally less than 100 based on either measured values or 210 ppb.

IV. B. Residential Exposure

IV. B. 1. Structural Fumigation

For residents living in treated homes after aeration, the acute MOEs were not calculated due to lack of exposure data. They were expected to be at least 100 since regulations were based on the 210 ppb for acute exposure.

IV. B. 2. Field Fumigation

For the 95th percentile exposure at the buffer zone perimeter, the acute MOE ranged from 89 (40 acres/80 lbs) to 131 (10 acres/80 lbs) (Table 6a). The interpretation of these MOEs is not as straight forward as those based on point estimates since they are based on a frequency distribution and on maximum air concentrations along the perimeter. When the MOE is less than 100 based on a 95th percentile value, it means that the reference concentration of 210 ppb was exceeded in less than 5% of the 7166 24-hour meteorological data sets and only along the portion of the buffer zone perimeter with the maximum methyl bromide air concentration. At the 90th percentile methyl bromide air concentration, all MOEs were at or greater than 100. At the 95th percentile, the MOEs were at least 100 (98 to 131) for 1 and 10 acres and all emission rates. For

20 and 30 acres, the MOEs were around 100 (96 to 104) with the exception of 91 and 93 for 80 lbs emission rate. For 40 acres, the MOEs were 89 to 95 for the specified emission rates.

IV. B. 3. Commodity Fumigation

The acute MOE for residents living near commodity fumigation facilities was 100 because the exposure was assumed to be 210 ppb (Table 6b). However, the MOEs were 39-78, 5-13, and 1, respectively, for short-term, subchronic, and chronic exposures based on 210 ppb as the average daily exposure levels.

IV. B. 4. All Uses

For residents living around methyl bromide uses, ambient air monitoring of 12 sites showed MOEs ranged from 695 to >80,000 for acute exposure, and from 409 to > 40,000 for short-term exposures (Table 6c). For 7-8 weeks of exposure, the MOEs for all sites were greater than 100 (range form 115 to 10512).

Table 3. Margins of exposure for occupational exposures to methyl bromide in field fumigations.^a

Type of Application	n ^b	Acute	Short-term	Subchronic	Chronic
a. Shallow-shank/ tarp / broadcast - (Noble plow, 10-12" injection, open cab and overhead fan for applicators; <i>Table B1,2,3</i>)					
Applicator	8	112	182	46	n/a
Copilot	7	86	121	31	n/a
Shovel-man	10	110	364	n/a	n/a
b. Shallow shank/ tarp/ bed - Scrapers (6-8" injection, conventional shank, copilot on raised platform in one case, scrapers/closing shoes and rollers to compress the soil after injection in the other case; <i>Table B9</i>)					
1. Raised platform					
Applicator	1	144	255	63	n/a
Copilot	2	111	197	49	n/a
2. Closing shoes					
Applicator	1	263	462	120	n/a
Copilot	2	69	121	31	n/a
c. Shallow shank/ tarp/ bed - Drip tape (12-14" injection; swept-back shank, first tractor forms bed, injects methyl bromide, and puts on drip tape; second tractor lays on tarp; <i>Table B10</i>)					
1. Tractor fumigation					
Driver	1	412	706	174	n/a
Applicator	1	256	444	112	n/a
Drip tape layer	1	176	632	n/a	n/a
2. Tractor with tarp					
Driver	1	3000	6000	1563	n/a
Copilot	2	339	600	156	n/a
d. Shallow shank/ tarp/ bed - Closing device (6-8" injection, swept-back shank, use of a closing device and compaction roller to compress the soil before tarp application; <i>Table B11</i>)					
Applicator	1	5250	12000	1563	n/a
Copilot	2	362	622	156	n/a
Shovel-man	2	21000	60000	n/a	n/a
e. Shallow shank/ tarp - Tarp removal study 1 (10-12" injection, Noble plow, tarp cut 5 days after fumigation and removed after 1 day of aeration; <i>Table B12</i>)					
Cutter	3	104	308	87	n/a
Puller	12	98	429	120	n/a
f. Shallow shank/ tarp - Tarp removal study 2 (10" injection, Noble plow, tarp cut 5 days after fumigation and removed after 1 day of aeration; <i>Table B13</i>)					
Cutter	1	152	324	92	n/a
Remover:tractor driver	1	20	42	12	n/a
Remover:basket-man	1	21	44	12	n/a
Remover:end puller	1	955	2000	521	n/a

Table 3. Margins of exposure for occupational exposures to methyl bromide in field fumigation (continued).^a

Type of Application	n ^b	Acute	Short-term	Subchronic	Chronic
g. Deep-shank/ non-tarp - Overhead fan (20-24" injection, open cab and overhead fan for applicators; <i>Table B6</i>)					
Applicators	2	75	132	33	n/a
Copilot	1	236	414	104	n/a
Cultipacker	1	116	203	n/a	n/a
h. Deep-shank/ non-tarp - Overhead fan and scrapers (20-24" injection, open cab and overhead fan for applicators, scrapers and press wheels to compress the soil after injection; <i>Table B6</i>)					
Applicator	1	202	353	92	n/a
Cultipacker	1	164	293	n/a	n/a
i. Deep-shank/ non-tarp - Enclosed cab (24" injection, enclosed cab for applicators, closing scrapers, second tractor equipped with either a disc or cultipacker; <i>Table B7</i>)					
1. Disc					
Applicator	1	130	231	58	n/a
Disc driver	1	22	40	10	n/a
2. Cultipacker					
Applicator	2	123	214	54	n/a
Supervisor	1	172	300	74	n/a
Cultipacker	2	339	600	n/a	n/a
j. Deep-shank/ non-tarp - Enclosed cab and scrapers (27" injection, enclosed cab for applicators, scrapers and rollers to compress the soil after injection; <i>Table B8</i>)					
Applicator	1	1615	3000	782	n/a
Cultipacker	1	1615	3000	n/a	n/a

^{a/} Margins of exposure were based on exposure levels in Table 19 of Volume I and human equivalents of the critical NOELs: acute, 40 ppm (adult equivalent of 21 ppm) for developmental toxicity in rabbits; short-term, 20 ppm (adult equivalent of 12 ppm) for neurotoxicity in rabbits; subchronic, 5 ppm (adult equivalent of 1.56 ppm) for neurotoxicity in dogs; and chronic, 0.3 ppm (adult equivalent of 200 ppb) for nasal epithelial hyperplasia/degeneration in rats.

^{b/} n=number of measurements.

Table 4. Margins of exposure for occupational exposures to methyl bromide in commodity fumigation.^a

Type of Application	n ^b	Acute	Short-term	Subchronic	Chronic
a. Greenhouse potting soil- hot gas method					
Tarp ventor	4	100	>100000	n/a	n/a
Tarp remover	4	100	85714	n/a	n/a
b. Fumigation of grain products					
Aerator (sea container/trailer)	3	100	27907	5210	667
Aerator (tarp)	3	100	>100000	156300	20000
Forklift driver (container/trailer)	3	100	1091	195	25
Forklift driver (chamber)	3	100	3000	521	67
c. Fumigation of dried fruit and tree nut products					
1. Raisins					
Fumigator	2	100	222	36	8
Aerator	2	100	300	47	11
Clear chamber	0	100	67	11	2
Stem picker	2	100	500	78	17
Forklift driver	1	100	4000	782	200
Hopper operator	1	100	750	120	25
2. Walnut processing facility					
Bulk packaging	2	100	414	56	n/a
Cleaning plant	12	100	67	9	n/a
Fumigatorium	3	100	160	21	5
Packaging	1	100	316	42	n/a
Vacuum chamber	0	100	67	9	n/a
Sorting	6	100	444	58	n/a
Special cracking	4	100	480	65	n/a
d. Fumigation and aeration at a brewery facility					
1. Applicator					
Entry to open canisters	4	100	1500	n/a	n/a
Area sample	1	100	1000	n/a	n/a
2. Aerator					
Aerator	2	100	1714	n/a	n/a
Area sample	2	100	245-414	n/a	n/a

^{a/} Margins of exposure were based on exposure levels in Table 20 in Volume I and human equivalents of the critical NOELs: acute, 40 ppm (adult equivalent of 21 ppm) for developmental toxicity in rabbits; short-term, 20 ppm (adult equivalent of 12 ppm) for neurotoxicity in rabbits; subchronic, 5 ppm (adult equivalent of 1.56 ppm) for neurotoxicity in dogs; and chronic, 0.3 ppm (adult equivalent of 200 ppb) for nasal epithelial hyperplasia/degeneration in rats.

^{b/} n=number of measurements.

Table 5. Margins of exposure for occupational exposures to ambient and area air sampling of methyl bromide in commodity fumigation facilities.^a

Type of Application	n ^b	Acute	Short-term	Subchronic	Chronic
a. Chambers (raisins)					
Chamber	1	100	160	25	6
Cage	1	100	261	41	9
Leak checker	2	100	n/a	n/a	n/a
Aeration	2	100	121	19	4
Clearing	2	100	308	49	11
Hopper area	2	100	1714	261	67
Stem picker area	4	100	522	82	18
b. Walnut processing facility					
1. Area samples					
Sorting line	2	100	500	n/a	n/a
2. Compliance monitoring					
Sorting line	0	100	67	9	n/a
Cello packaging	0	100	67	9	n/a
Bulk packaging	0	100	67	9	n/a

^{a/} Margins of exposure were based on exposure levels in Table 21 in Volume I and human equivalents of the critical NOELs: acute, 40 ppm (adult equivalent of 21 ppm) for developmental toxicity in rabbits; short-term, 20 ppm (adult equivalent of 12 ppm) for neurotoxicity in rabbits; subchronic, 5 ppm (adult equivalent of 1.56 ppm) for neurotoxicity in dogs; and chronic, 0.3 ppm (adult equivalent of 200 ppb) for nasal epithelial hyperplasia/degeneration in rats.

^{b/} n=number of measurements.

Table 6. Margins of exposure for residential exposures to methyl bromide from living near field or commodity fumigation activities.^a

a. At buffer zone perimeter of fumigated fields- Acute exposure						
Field size	Cumulative frequency	Margins of exposure for different emission rates (80-320 lbs methyl bromide /acre-day)				
		80	160	200	225	320
1 acre	0.9	146	138	139	140	142
	0.95	131	121	121	121	122
10 acres	0.9	111	120	124	129	152
	0.95	98	103	106	110	129
20 acres	0.9	106	115	118	119	122
	0.95	93	99	101	102	104
30 acres	0.9	103	115	115	115	113
	0.95	91	99	98	98	96
40 acres	0.9	100	110	111	110	107
	0.95	89	95	95	94	92
b. Near commodity fumigation facilities						
Type of Application	Acute	Short-term	Sub-chronic	Chronic		
low range	100	78	13	1		
high range	100	39	5	1		

Table 6. Margins of exposure for residential exposures to methyl bromide from living near field or commodity fumigation activities (continued).^a

c. Ambient monitoring in three California counties					
Sites in California	Daily (ppb)	Weekly (ppb)	7-8 weeks (ppb)	Chronic	
Monterey					
Chualar School, Chualar	9292	4294	1371	n/a	
La Joya Elementary School, Salinas	1135	631	233		
Oak Avenue School, Greenfield	17355	7625	2282		
Pajaro Middle School, Watsonville	695	409	115		
Ambient Monitoring Station, Salinas	3404	2229	690		
Santa Cruz					
Salsepuedes Elementary School, Watsonville	1721	940	340		
Kern					
Ambient Monitoring Station, Bakersfield	37770	13807	4672		
Cotton Research Station, Shafter	827	1264	409		
Mettler-Fire Station, Mettler	87866	42945	10512		
Mountain View School, Lamont	80153	35897	9598		
Shafter-Walker Ambient Monitoring Station	5276	3415	1115		
Vineland School District, Bakersfield	71918	38674	8919		

^{a/} Margins of exposure were based on exposure levels in Table 22 of Volume I and human equivalents of the critical NOELs: acute, 40 ppm (adult equivalent of 21 ppm) for developmental toxicity in rabbits; short-term and weekly, 20 ppm (child equivalent of 7 ppm) for neurotoxicity in rabbits; subchronic and 7-8 weeks, 5 ppm (child equivalent of 0.88 ppm) for neurotoxicity in dogs; and chronic, 0.3 ppm (child equivalent of 100 ppb) for nasal epithelial hyperplasia/degeneration in rats.

V. RISK APPRAISAL

The limitations and uncertainties associated with the risk characterization of methyl bromide inhalation exposure have already been discussed in the 2002 RCD. The following is a revision to that discussion based on the change in the critical NOEL for subchronic exposure.

V. A. Hazard Identification

For acute inhalation exposure to methyl bromide, the critical NOEL was based on developmental effects observed in rabbits with the assumption that methyl bromide will also cause developmental toxicity in humans. There are no data to support or refute this assumption. The reference concentration (210 ppb) for this NOEL was only 1.5-fold lower than that for neurotoxicity in humans (350 ppb). The endpoints for the critical short-term and subchronic exposures were based on neurotoxicity in the pregnant rabbit and dogs, respectively. There were uncertainties associated with the use of hyperplasia/degeneration to the nasal cavity of rats as the endpoint to evaluate chronic inhalation toxicity. One uncertainty was the interspecies variability in the nasal cavity between rodents and humans. No additional information is available on the pharmacokinetics of methyl bromide in the nasal cavity epithelium of animals and humans, which would permit additional consideration of this endpoint.

In the 2002 RCD, both the subchronic and chronic NOELs were estimated from the LOEL, the lowest dose tested. With the use of the experimental NOEL of 5 ppm (Schaefer, 2002) for subchronic exposure in this Addendum, the uncertainty associated with the extrapolation of a ENEL in the 2002 RCD was eliminated. In addition, there is general consensus from both internal and external scientific reviews that 5 ppm was very close to the actual NOEL for subchronic exposure (see reviewers' comments in the Appendices). For chronic exposures, the uncertainty with the extrapolation remained, as the estimated NOEL was 0.3 ppm based on a LOEL of 3 ppm for nasal epithelial hyperplasia and degeneration in the rat, using an uncertainty factor of 10. The mildness of the lesion at the LOEL suggested that an UF of less than 10 might be sufficient to estimate the NOEL from the LOEL.

V. B. Exposure Assessment

Since there was no change in the exposure assessment, the limitations in the estimated exposures remained the same as discussed in the 2002 RCD. The major limitation in the worker (all uses) and residential (commodity fumigation) exposure assessment was that data were not available for many scenarios, as some acute exposures were assumed to be or limited to 210 ppb. The use of 210 ppb exposures might be over- or underestimation of actual acute exposures. Of the available data, there were many deficiencies in the overall database and they included: small sample size, incomplete report, and short monitoring period. Potential areas of underestimation were the assumptions of performing a single work task per day and that no overtime was worked. One area of overestimation was the use of a 50% recovery value to correct measured data.

For residential exposure to field fumigation, there were also uncertainties in the determination of the maximum methyl bromide air concentration distribution along the buffer zone perimeter of fumigated fields. These uncertainties included: the precision and accuracy of

the sampling and analytical methods, influence of environmental factors on air concentrations, application variability, use of default weather conditions, and use of default assumptions in estimating air concentrations associated with overlapping applications. Actual exposure may be underestimated or overestimated because of these uncertainties.

V. C. Risk Characterization

There is also no change in the use of the default uncertainty factor of 10-fold each to address the extrapolation of no-effects from experimental animals to humans (interspecies), and accounting for intraspecies variations. The additional study of neurotoxicity in dogs (Schaefer, 2002) did not change the previous conclusion that an additional uncertainty factor, as described under the Food Quality Protection Act, was not needed. The result from the study did not provide any evidence for potential increased sensitivity for infants and children.

VI. CONCLUSION

The human health risk from subchronic inhalation exposure to methyl bromide was reevaluated in this Addendum. This reevaluation was conducted because of results from a new inhalation toxicity study in dogs. After consideration of comments from internal and external scientific reviews, DPR determined that the critical subchronic NOEL should be increased to 5 ppm from 0.5 ppm used in the 2002 RCD. Since the subchronic exposure scenario was not considered in the risk characterization documents for dietary and aggregate exposures, this change in the NOEL does not have any impact to the conclusions of those documents.

For some exposure scenarios, the revised MOEs for subchronic exposure were greater than the benchmark of 100 used to determine the acceptability of the exposure. However, there remained some occupational and residential scenarios with MOEs of less than 100 and mitigation measures might be warranted. The risk characterization in the 2002 RCD and in this Addendum showed that with structural fumigation, the acute MOEs for workers and residents remained at least 100, based on restrictions in the DPR regulations. However, data are still needed to estimate actual exposures for acute and short-term exposures for workers and residents. For field fumigation, the acute MOEs and short-term MOEs remained at less than 100 for disc drivers (deep shank injection), and tractor drivers and basket-men in tarp removal (shallow shank injection with Noble plow). For subchronic exposure, the MOEs for many exposure scenarios (applicators, copilots, disc drivers, and tarp removers) remained less than the benchmark of 100 since they were less than 10 using the lower NOEL in the 2002 RCD. The MOE for workers at adjacent fields was assumed to be 100 since they work outside of the buffer zone. For residents living at the buffer zone perimeter of fumigated fields, the acute MOEs were generally around 100 for the 95th percentile exposure. The acute MOEs were generally greater than 100 at the 90% percentile exposure.

For commodity fumigation, the acute MOEs for workers involved in fumigation remained at 100 because DPR regulation set work hour restrictions to limit the maximum exposure at 210 ppb, and no new data have been submitted. The short-term MOEs were greater than 100 for all work tasks based on actual measurements with few exceptions. The subchronic and chronic MOEs remained at less than 100 for many scenarios in the fumigation of dried fruit, tree nut products, and raisins, and in the processing of treated walnuts. For residents living near fumigation facilities, the MOEs for all durations were based on 210 ppb used for acute exposure, and not based on actual measurements. The MOEs were between 1 and 78 for short-term, subchronic and chronic exposures. However, the MOEs were greater than 100 for ambient air exposures at all school sites for all durations of exposure.

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Appendices

A. Toxicology Summary

B. Internal Review by Dr. T. Kellner, Department of Pesticide Regulation

C. External Review by Dr. K.E. Pinkerton, University of California at Davis

D. Memorandum from Dr. G. Patterson to Dr. T. Jones, Department of Pesticide Regulation

E. Selection of Critical NOEL for Methyl Bromide Subchronic Inhalation Toxicity

F. External Review by Dr. J. Last, University of California at Davis

(Note: The format of the electronic versions of these Appendices may be slightly different than the original version. The texts for both versions should be identical. The original version of each Appendix is available upon request to the Department of Pesticide Regulation.)

Appendix A

Toxicology Summary

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; March 5, 1999; and Jan. 27, 2003

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 onco-genic effect for inhalation exposure.

⁴ Under SB950, this category is for acute delayed neuropathy testing in hens for agents with anti-cholinesterase 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: T030127

Revised by: Joyce Gee (1/27/03)

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

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 spongio-sis hepatitis 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 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 re-view 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) ≥ 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 CrI: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 Pharma-co 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).

123-172 143942 □A Four Week Dietary Range-Finding Toxicity Study of Methyl Bromide in Rats□ (Tompkins, E.C.; laboratory study number: WIL-49013; August 11, 1995). Apparently, this study was the basis for the selection of 250 ppm as the sole test dose in the two-week dietary range-finding toxicity study (record 162360). For 28-30 days, 15 CrI:CD□BR rats/sex/dose were administered methyl bromide presented as microcapsules dispersed in feed. The microencapsulated material appears to have been the 0.48% methyl bromide material used in the two-year study (record 158746). The doses tested in record 143942 were: 0 ppm (basal diet), 0 ppm (placebo microcapsules), 0.1 ppm, 1.0 ppm, 10 ppm and 100 ppm. The highest dose represented nominal doses that ranged from 6 to 9 mg/kg/day, depending on the test week and sex. All rats survived to scheduled sacrifice. No significant effects were observed in the following areas: clinical observations; hematology; serum chemistry; necropsy; absolute organs weights and organs weights relative to bodyweight; and histology (using an abbreviated selection of organs). The only statistically significant finding was decreased food consumption in the 100 ppm male group for each of the four test weeks. Also, absolute bodyweight of the 100 ppm male group was 96% of the values for the placebo-microcapsule male group for each of the four test weeks; for the 100 ppm female group, absolute bodyweight was 95-96% of the values for the placebo-microcapsule female group for test weeks 2 through 4. **NOEL (4 weeks) > 100 ppm.** Record 143942 is also notable for the following. First, both sexes received ~30 grams of feed daily. Inspection of the individual data indicates that in test weeks 3 and 4, there were occasionally males in the 0 to 10 ppm groups whose mean daily feed consumption was \geq 29 grams. This is noted because rats that were eating all of their ration may have undergone a mild form of feed restriction (i.e., these rats may have consumed more feed if given the chance to eat ad libitum). Such feed restriction also occurred in two later studies: records 162360 and 158746. Second, the determination of methyl bromide in the feed was similar to that used in the two-year study (record 158746): a □relative□ assay was used (the standards were feed fortified with the same microcapsules that were used to prepare the test feeds); and the headspace analysis involved heating for 15 minutes at 100°C preinjection. It was indicated that as part of the quantitation of methyl bromide, its conversion to methyl chloride was taken into account (note: chromatograms from the analyses done for record 143942 appear in Exhibit A1 in record 160305; these chromatograms indicate that there was significant methyl chloride production with this method). Third, no loss of methyl bromide was observed in feed preparations stored at room temperature for 16 or 24 hours. By contrast, ~30% loss was reported in record 158746 in comparable studies with this same microencapsulated material. **Supplemental information.** (Rinkus, 12/3/99).

123-207 165140 This record was the response from the Registrant to the March 20, 1998 re-view of record 158746. The initial section was a 6-page narrative addressing: the bioavailability of methyl bromide when using microencapsulated material; and specific items discussed in memo-random M980512, dated May 12, 1998, from the DPR MT reviewer (Dr. Rinkus) to Gary Patter-son (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 Fum-igated 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. **Supplementary information. No worksheet.** (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 CrI: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 worksheet.** (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].

123-109 087805 "Histopathology of Acute Toxic Responses in Selected Tissues from Rats Exposed by Inhalation to Methyl Bromide," (Hurt 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 cytoplasm; and a diffuse granule cell degeneration without the edematous cytoplasm); 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). **Supplementary 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 CrI: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 after-noon 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).

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 un-acceptable 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 hand-written 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).

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. Supplementary 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 ≥ 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. Histo-logical 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 fasciculata, 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 ex-posure day 14; but no findings were made for the 50 ppm group in postexposure examinations, in-cluding 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).

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).

123-212 187459 "A 6-Week Inhalation Toxicity Study of Methyl Bromide in Dogs" (Schaefer, G.J.; study number: WIL-440001; 5/16/02). Methyl bromide (100% purity) was administered to beagle dogs (4 dogs/sex/treatment level) by whole body inhalation at 7 h/d, generally 5 d/week (exceptions: ≤ 3 days in initial week; 6 days in penultimate week; ≤ 4 days in final week), for at least 30 exposure days. Treatment levels were 0, 5, 10 and 20 ppm. Dose levels were chosen in order to test whether 5 ppm, at 7 h/d for 30 exposure days, would result in neurological changes, as had been suggested in the previous dog inhalation study (record 132821). During study weeks -2, -1, 2, 4 and 6, Functional-Observational-Battery (FOB) testing was performed, followed by motor-activity measurements using an automated apparatus. Due to the experimental design, the cumulative number of exposure hours on a weekly basis before the FOB and motor-activity testing were conducted in study weeks 2, 4 and 6 may have ranged from 14 h to 35 h within each group. If the cumulative number of hours of exposure in the week of the FOB-motor activity testing is important for observing neurological effects in such testing, this design may not have been optimal (that the design also may have been unavoidable is appreciated). After exsanguination, in situ perfusion with para-formaldehyde and glutaraldehyde was performed to allow for the histological examination of nervous-system tissues in accordance with USEPA neurotoxicity guidelines (OPPTS 870.6200--Neurotoxicity Screening Battery-August, 1998). No deaths occurred during the study and observed effects were mild in comparison to the worst produced in record 132821. There were no indications of a treatment effect on the following: bodyweight, feed consumption, rectal temperature, and fixed spleen weight (absolute or relative to bodyweight). All dogs underwent necropsy; no treatment effects were observed. Histological examinations were limited to H & E preparations of nervous-system tissues from high-dose and negative-control dogs (both sexes); no treatment effects were observed. In the FOB testing, absence of the proprioceptive-placing response during table-top measurements was noted in two consecutive sessions with a male from the 10 ppm group, in three consecutive testing sessions with a male from the 20 ppm group, and in the first testing session with a female from the 20 ppm group. Pretesting data and historical negative-control data indicate that it is rare for untreated dogs not to exhibit this response, especially in a repetitive manner. In the testing using a motor-activity-measurement apparatus, there was no obvious treatment effect but supplemental information is needed to complete the evaluation of these data. Clinical-examination findings included the following: emesis by two 20 ppm females at the end of study week 4; clear discharge from eye(s) by two 20 ppm males and possibly a 20 ppm female; and feces-related findings (soft feces, mucoid

feces, mucoid feces with blood, and [or] diarrhea) in four 20 ppm males, two 20 ppm females, two 10 ppm males and one 10 ppm female. One 5 ppm male exhibited twitching or tremors on three separate days. Also, three 5 ppm males that had not been observed to vomit during the pretest period did so (once each) while on test; in one case, emesis occurred after the first or second day of exposure, when there may have been difficulties in controlling methyl bromide release into the inhalation chamber. Regarding the 5 ppm male with twitching and tremors, although the researchers considered this animal to be afflicted with idiopathic febrile necrotizing arteritis, the basis for this diagnosis is unclear and supplemental information is needed to assess this. **LOAEL = 5 ppm.** Major deficiencies include: 1) positive-control data regarding the FOB testing, motor-activity measurements and nervous-system histology were either inadequate or not provided; 2) the histological evaluation of the nervous-system tissues did not include the use of special stains and it is unclear whether the findings from the previous dog inhalation study (record 132821) were used to guide the histological examination in this study; and 3) some methods, data and a protocol deviation regarding the male presumed to be exhibiting idiopathic febrile necrotizing arteritis were not provided. **Supplemental information.** (Rinkus, 8/16/02).

Note: Because of the pivotal role of this study for determining the NOEL for subchronic exposure for risk assessment, it was reviewed by additional scientific staff (including the senior scientific staff) and by external peer-reviewers at the University of California, Davis. The consensus was that the 5 ppm exposure should be considered the NOEL, although this conclusion is judgmental and reasonable people may differ in the interpretation of the results. Supporting documentation is on file. (Gee, 1/27/03).

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 re-viewed (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 (work-sheet 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. It 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

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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 re-cord 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).

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, histological 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--Reexamination 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 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).

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 un-treated 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 toxicity, 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 degenerative 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 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 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

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Toxicology, 1:129-135, 1979]). Other parameters also were decreased in the F1 90 ppm females (parameters IIIh and IVb) or F1 males (parameters IIIa and IIIc). 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).

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 IIIh and IVb) or F1 males (parameters IIIa and IIIc). 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, Toxicogenics 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 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, morphology, 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).

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 re-view 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] (Kane-da, 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 body-weight 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/sternae; 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. **Un-acceptable.** 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 neuro-toxicity (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: omphalocele (80 ppm group, part I); hemorrhaging with or without hydrops (80 ppm, parts I & II); retroeso-phageal right subclavian artery (80 ppm group, part I); gall bladder agenesis (80 ppm, parts I & II); and fused sternbrae (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 sternbrae; 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, re-troesophageal right subclavian artery, gall bladder agenesis, fused sternbrae 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 data 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 laboratory; 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 embryoletality 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] (Kane-da, 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 body-weight 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/sternbrae; 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).

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).

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 maximum 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/m³ (1271 ppm); the estimated concentration of methyl bromide in the nutrient broth was 250 µM; 2) TA100, 1900 mg/m³ (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/m³ (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 reported 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/m³ (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 fumigant 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," (Djalali-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 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 $\mu\text{g}/\text{kg}$ bodyweight. Bacterial mutagenicity was observed at test concentrations of ≥ 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. **UNACCEPT-ABLE**. 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).

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 tab-let 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 ex-changes (SCEs). Twenty-five second division metaphase cells were scored per mouse. In the ini-tial 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 expo-sure period.** In the 12-week study, no increase in the frequency of SCEs/cell was observed for either sex. **NOAEL > 120 ppm for a subchronic exposure.** While it may be unexpected that a re-sponse would only be seen in the initial testing, without replicate testing or other supplemental in-formation, 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 mi-totic 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., Scot-land; 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. UNACCEPT-ABLE but upgradeable upon submission of a more detailed explanation of how the cells were ex-posed to test material, the number of cultures per treatment level, and cytotoxicity 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 concen-trations 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 categorized in terms of the frequencies of abnor-mally 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 up-graded 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 O⁶-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

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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).

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 al-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).

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 \square 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 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 \square 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 \square 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 activity; 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-1 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; BRRRC 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; BRRRC 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; BRRRC 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 in-tended to train 7 people to perform a functional observation battery (FOB), including the two ob-servers 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 in-formation.** (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 micro-wave 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 pre-synaptic 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 nor clonidine were administered before the locomotor activity was recorded. The DPR MT review-er'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 ex-posures 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 \geq monkeys $>$ guinea pigs \geq 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. (Kishiya-ma, 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).

RESIDUE STUDIES

Note: Record 126281 demonstrated that methyl bromide is readily converted to methyl chloride in the

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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 matrices 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 drinking-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).

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 1/92) 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 1/27/03 have been considered in this SUMMARY OF TOXICOLOGY DATA. The following also have been received but have not been reviewed:

- 1) 123-173 143943 This is a copy of a memorandum 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."
- 2) 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.
- 3) 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).

Appendix B

Internal Review by Dr. T. Kellner, Department of Pesticide Regulation

TO: Gary Patterson
Supervising Toxicologist
Medical Toxicology Branch

FROM: Thomas Kellner
Staff Toxicologist
Medical Toxicology Branch

DATE: July 2, 2002

SUBJECT: Methyl Bromide (Schaefer) Neurotoxicity Study in Dogs

Neurotoxicity screening batteries such as the FOB have been used to test a wide range of economically important chemicals and environmental contaminants for neurotoxic potential. Unlike the carbamate and OP pesticides that have a well-documented mechanism of action, most chemicals subjected to neurotoxicity screening batteries represent virtual unknowns as far as specific neurotoxicity.

The neurobehavioral data collected from screening studies should be organized in such a way as to separate chemicals with specific neurotoxic action from those that produce neurobehavioral changes that are secondary to systemic toxicity. One way to delineate compounds that can be classified as specifically “neurotoxic” is based on clear evidence of neurobehavioral changes in multiple endpoints (i.e., effects related to specific areas of the central or peripheral nervous system by functional domains, as prescribed by V. Moser). In this way, clear evidence of neurotoxicity can be differentiated from the spurious, unrelated measures in the FOB.

Alternatively, criteria for delineating a neurological threshold for risk assessment, especially for chemicals which are known to be neurotoxic (e.g., OP and carbamate pesticides), could be somewhat different. Endpoints in the FOB that may occur in isolation or in relatively few clusters (i.e., not quite sufficient for establishing a chemical as a neurotoxicant) may very well be pertinent for use in risk assessment. The endpoint selection for risk assessment should still consider the dose-related pattern and the biological plausibility based on the chemical's mode of toxicity. If isolated FOB findings are within a group of neurotoxic manifestations characteristic for a known neurotoxicant, then they should be considered pertinent for characterizing a NOEL for risk assessment.

In the Schaefer six-week methyl bromide study in dogs (vol. 123-212: 187459), isolated changes in proprioceptive-placing response (absence) in one male at 10 ppm and one animal per sex at 20 ppm were the only noteworthy FOB changes reported. Proprioceptive positioning reaction is a type of postural reaction, and involves more complex neural circuitry than simple reflexes, as explained below.

Reflexes are involuntary and relatively stereotyped response to a specific sensory stimulus. Reflexes are frequently named according to the body part stimulated during the test (eg., patellar and palpebral reflex). In practical terms, reflexes that can be tested during the FOB include cranial nerve reflexes (palpebral, pinna and pupillary reflex) and spinal reflexes (flexor, grasp and extensor thrust reflex), with the measurement of each requiring a prescribed procedure. The simple neuronal circuits that mediate reflexes can then be influenced by the brain, with the brain coordinating the action of several reflex circuits to generate more complex behaviors called reactions (e.g., placing reaction). For example, postural reactions are complex responses that maintain an animal's normal, upright position under conditions of shifting loads. Abnormalities of complex postural reactions (e.g., hopping) do not provide anatomically precise information about neurologic abnormalities. Reflex tests, which are more limited in scope, can provide such information. Deficits in postural reactions are often used as screening tests of function, and changes in reflexes are used to characterize/localize functional effects in greater detail. Reactions include postural reactions, proprioceptive positioning and righting reactions (surface righting and aerial righting reaction).

Because the brain will coordinate the action of several reflex circuits to generate more complex reaction behaviors such as proprioceptive positioning, one would expect that some changes in spinal reflexes (flexor, grasp and extensor thrust reflex), motor strength (wheel barrowing, hemistanding/hemiwalking parameters), open field or table-top observations would also be noted in the FOB sessions where proprioceptive positioning changes were occurring (i.e., according to Moser, deficits revealed during proprioceptive positioning are generally confirmed by deficits in other postural reactions). This situation did not occur in the case in the Schaefer study. The most outstanding example of proprioceptive positioning change in the study was in a high-dose, 20 ppm male (no. 8723) at week 2, 4 and 6. None of these observations were accompanied by changes in the reflex or motor strength parameters mentioned above. Specifically, no changes were noted for pinch reflex (front and back legs), righting reflex, triceps reflex, patellar reflex, posterior extension thrust, wheel barrowing, hemistanding, time to first step, or overall gait.

Although proprioception is a sensory function, adequate motor strength is required for performance by the subject. The sensory nerves/regions tested involve the large sensory fibers to the skin and joints, dorsal columns (gracilus, cuneatus) and spinomedullothalamic pathway and the somatosensory cortex. The motor response is initiated in the brain and is transmitted through the spinal cord. Motor nerves to somatic muscle of the hip, knee, and foot are required. One could argue that the deficit in proprioceptive positioning being demonstrated by methyl bromide was of a sensory nature and didn't involve a motor component. This situation is unlikely, in that all of the remaining reflexes and reactions involve a sensory component and did not show a complementary effect.

This lack of complementary reflex findings tended to support the argument that the proprioceptive positioning changes were incidental findings. The only other animal in this dose

group to show this change was a female during the week 2 observation only. In addition, no significant microscopic changes were reported in the peripheral nerves or spinal cord in these animals. Because of a lack of clear dose response, the lack of accompanying changes in reflex responses and the isolated nature of the proprioceptive positioning change in the female at 20 ppm, it is unclear what weight, if any, should be given to this finding.

Schaefer found the changes proprioceptive positioning important enough to specifically mention them in the results section, but also tried to mitigate their impact. Although the thrust of my discussion is also on the side of calling these findings incidental, I could understand why a Medical Toxicology reviewer would flag these changes as compound-related for health-protective reasons. It is a difficult decision, but my overall impression is that the proprioceptive positioning changes are incidental.

cc: Joyce Gee
Lori Lim

Appendix C

External Review by Dr. K.E. Pinkerton, University of California at Davis

July 19, 2002

Gary Patterson, Supervising Toxicologist
Medical Toxicology Branch
Department of Pesticide Regulation
State of California
1001 I Street, P.O. Box 4015
Sacramento, CA 95812-4015

Dear Dr. Patterson:

Thank you for the opportunity to review the abbreviated version of the Schaefer final report (2001) "A six-week inhalation toxicity study of methyl bromide in dogs." The evaluation of the Schaefer report by Dr. Janice Chambers and the memorandum of Dr. Thomas Kellner on neurotoxicity screening batteries and proprioceptive positioning were also helpful to review. I agree with Dr. Chambers that the Schaefer Study (2001) to examine the effects of methyl bromide in dogs is a more complete study than the Newton Study (1994). The study by Newton was not designed to test neurotoxicity, but rather to serve as a range-finding study to determine tolerable exposure levels for a long-term study using methyl bromide. As a result, relatively small numbers of animals were used. In contrast, the Schaefer report used four groups of dogs, with four male and four female beagles in each group. Treatment regimens consisted of exposure to methyl bromide at 5, 10, and 20 parts per million (ppm), as well as a fourth group exposed only to filtered air. Exposures were done for 7 hours per day, 5 days per week, for 6 weeks. Daily clinical examinations were performed, while functional observational battery (FOB) and motor activity assessments were done two times prior to the onset of the study and following 2, 4, and 6 weeks of exposure for all groups. A complete necropsy evaluation was also performed at the end of the study for all treatment groups and controls. Therefore, the recommendation of Professor Chambers to use the Schaefer study to more accurately set the standard for methyl bromide rather than the Newton study is highly appropriate. I would agree that the Schaefer study provides a more definitive means to begin to set health safety standards for inhalation exposure limits to methyl bromide.

Exposure methods, measurement of exposure concentrations, and anatomic pathology completed were all done in an appropriate and carefully executed manner for the Schaefer report. However, some concern not expressed by Dr. Chambers or by the Schaefer report remains in my mind for the findings of proprioceptive-placing in this study. The proprioceptive-placing described in the Schaefer report and by Dr. Kellner is a measure of sensory function that requires adequate motor strength for its performance. The findings of the study demonstrated at an exposure of 20 ppm methyl bromide one male animal had the absence of proprioceptive-placing during weeks 2, 4, and 6. In this same exposure group, one female dog was also found to lack proprioceptive-placing at 2 weeks. The study (i.e., investigators) further noted that one male dog exposed at 10 ppm had the absence of proprioceptive-placing at 2 and 4 weeks.

Complete necropsy of all animals failed to demonstrate any appreciable differences in anatomical signs of neurotoxicity between the three exposure groups and the filtered air controls. In spite of inconsequential anatomical findings, it remains unclear why three animals exposed to methyl bromide at concentrations of 10 to 20 ppm would develop the absence of proprioceptive-placing during a portion or the entire period of the exposure period. The study design involved testing all animals two times prior to the onset of the experiment. Since all animals used in the study demonstrated no prior signs of the lack of proprioceptive-placing, it would seem that a loss of proprioceptive-placing within three animals exposed to any concentration of methyl bromide should raise concern regarding potential problems that may exist with exposure to methyl bromide at these concentrations. These findings are particularly disturbing based on (1) no control animals experienced lack of proprioceptive-placing, and (2) the effects of methyl bromide for this assay follows a dose response pattern (please see table below).

	<u>Lack of Proprioceptive-placing</u>			
	<u>Control</u> <u>Filtered Air</u>	<u>Methyl bromide (ppm)</u>		
		5	10	20
Pre-test 1	0/8	0/8	0/8	0/8
Pre-test 2	0/8	0/8	0/8	0/8
Week 2	0/8	0/8	1/8	2/8
Week 4	0/8	0/8	1/8	1/8
Week 6	0/8	0/8	0/8	1/8

One male dog exposed to 5 ppm of methyl bromide with idiopathic febrile necrotizing arteritis probably represents a random occurrence since this disease has been described repeatedly in beagle dogs, albeit the syndrome represents a rare condition. The conclusion I would draw from the Schaefer study is different from that of the scientists at WIL Research Laboratories and Dr. Chambers. In my opinion, the findings would more strongly support a No Observable Adverse Effects Level (NOAEL) should be assigned at 5 or 10 ppm rather than 20 ppm. Why wait for anatomical changes to conclude a neurotoxic effect of methyl bromide in dogs (or humans)?

Thank you again for the opportunity to review this document and the accompanying opinion statements.

Sincerely

Kent E. Pinkerton, Ph.D.
Professor of Anatomy, Physiology, and Cell Biology
School of Veterinary Medicine
Director, Center for Health and the Environment
University of California, Davis

Appendix D

**Memorandum from Dr. G. Patterson to Dr. T. Jones,
Department of Pesticide Regulation**

TO: Tobi Jones
Assistant Director
Division of Registration and Health Evaluation

FROM: Gary Patterson
Supervising Toxicologist (Managerial)
Medical Toxicology Branch

DATE: August 30, 2002

SUBJECT: Methyl Bromide Six-Week Inhalation Study with Dogs

Title: A 6-Week Inhalation Toxicity Study of Methyl Bromide in dogs
Author: G. J. Schaefer
Laboratory: WIL Research Laboratories, Inc., Ashland, Ohio
Date: May 16, 2002
In: Document 123 - 212, Record 187459

The study was conducted for the specific purpose of evaluating the neurotoxicity of methyl bromide in dogs by the inhalation route when exposure was continued for six weeks. Doses were selected based on previous data and were 0, 5, 10 and 20 ppm, with 4/sex/dose group. The study has been evaluated as supplemental data.

Attached is a detailed review of the above study prepared by Stephen J. Rinkus, Ph.D., Staff Toxicologist with the Medical Toxicology Branch, Department of Pesticide Regulation. He has assigned a Lowest Observed Adverse Effect Level (LOAEL) of 5 ppm to the study based, in part, on the tremors and twitching seen in one of eight dogs at that exposure level. This male dog (no. 8738) was described by the author (G.J. Schaefer) of the study report as diagnosed with Idiopathic Febrile Necrotizing Arteritis (IFNA), or beagle pain syndrome. The review describes the conduct of the study, deficiencies in the conduct and reporting of the study as noted by Dr. Rinkus, and the results, summarized from the document submitted by the Alliance of the Methyl Bromide Industry.

Because of the potential pivotal role of the study in risk assessment, the senior scientific staff of the Medical Toxicology Branch also has evaluated it. The results of the study have been discussed and, by consensus (although not unanimous), it was determined that the 5 ppm exposure should be the No Observed Adverse Effect Level rather than the LOAEL, with a number of areas of uncertainty, as outlined below. All staff agreed that 10 and 20 ppm demonstrated treatment-related effects. The determination of 5 ppm as a NOEL is based on the following:

1. No tremors or twitching were seen in the other 7 dogs at 5 ppm or in the 10 or 20 ppm exposure groups (although both of these signs have been reported at higher exposures of methyl bromide). The study did not show a dose response for these signs.
2. The signs of tremors and twitching were seen in a single dog at 5 ppm and that dog was diagnosed as having Idiopathic Febrile Necrotizing Arteritis and, therefore, may have been compromised. The signs were seen late in the study. Staff questioned whether this dog should be removed from consideration.
3. The lack of proprioceptive placing was not seen at 5 ppm during the FOB.
4. Disturbances of the gastrointestinal tract were reported primarily at 10 and 20 ppm as soft or mucoid feces (tainted with blood on occasion) and/or diarrhea with much lower incidences at 5 ppm and in control animals.

Areas of uncertainty in the study:

1. The diagnosis of Idiopathic Febrile Necrotizing Arteritis was not well substantiated in the report. No systemic hematology or histopathology was done on other dogs in the study to determine if similar findings of arteritis occurred. Rectal temperatures were taken biweekly and from those recorded, only at one interval was the temperature of dog 8738 marginally elevated above the normal range. No findings related to “pain” were recorded during the Functional Observation Battery (FOB). Proprioceptive placing was reported as normal. No histological changes were seen in the heart or coronary arteries.

No historical control data for IFNA from the supplier of the dogs, Ridglan Farms, were provided to substantiate the incidence as “rare”.

2. There were problems in generating the exposure atmospheres early in the study, with indications that the concentrations were not uniform and, at times, not close to target (based on the mean and one standard deviation for a chamber for a given day). The possibility, therefore, exists that acute daily exposures may have been substantially higher than the target, as discussed by Dr. Rinkus.
3. It was unclear how many dogs were in a given chamber on several occasions, as outlined in the evaluation of Dr. Rinkus.
4. The doses selected were quite close so that any variation in exposure would tend to make a dose response for any effect more difficult to demonstrate.

5. There were no pretest individual data for clinical signs.
6. There were no positive control data for the FOB included in the report and details regarding the staff conducting the test were not provided.
7. There were no positive control data for the neurohistopathology portion of the study. Although *in situ* perfusion was used for better observation of the nervous tissue, recommended specialty stains were not used.

CC: Joyce Gee, Peter Leung, Keith Pfeifer, Jay Schreider

Appendix E

Selection of Critical NOEL for Methyl Bromide Subchronic Inhalation Toxicity

(Note: This document was submitted to Dr. Last for review. It is referred to as the Addendum in Dr. Last's comments- in Appendix F. The page numbering noted in the Table of Content is for the original document).

Selection of Critical NOEL for Methyl Bromide Subchronic Inhalation Toxicity

**Medical Toxicology Branch
Department of Pesticide Regulation
California Environmental Protection Agency**

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I. Introduction

In 1999, the Department of Pesticide Regulation (DPR) completed a draft Risk Characterization Document (RCD) to address human inhalation exposure to methyl bromide in California (DPR, 1999). The draft RCD was reviewed by a Subcommittee of the National Research Council (NRC, 2000). Overall, the NRC agreed with the risk characterization of methyl bromide. However, the NRC was concerned that the toxicity endpoints used to calculate the risk might have been too conservative while the exposures might have been underestimated due to limitations in the available databases. The NRC recommended additional toxicology studies and exposure monitoring to better characterize the risk of human exposure to methyl bromide.

This document addresses one aspect of the risk characterization, the subchronic inhalation toxicity of methyl bromide. In the draft RCD, the critical No-Observed-Effect (NOEL)³ was selected from a dog study (Newton, 1994a). In this study, two dogs from a 5-ppm group, the lowest dose tested, were observed to be less responsive by an animal neurologist. This dose was considered as the Lowest-Observed-Adverse-Effect Level (LOAEL) for the study and a default extrapolation factor of 10 was used to estimate the NOEL, or 0.5 ppm. The NRC in the review of the RCD consider the effect in these two dogs as equivocal⁴ because of the “lack of dose-response curve at the lower dose levels, the observation of depressed activity in two of eight dogs outside the standard protocol procedures, and the low number of replications (NRC, 2000).” At the same time, the NRC concluded that it was reasonable to use the observation as the basis for the critical NOEL considering the seriousness of the neurotoxicity observed in humans and the potential long-term nature of the neurological effects. The NRC also agreed with the use of the 10-fold factor for the extrapolation of a LOEL to a NOEL. To clarify the neurotoxicity finding, the NRC recommended that a new subchronic toxicity study be conducted. In 2002, the registrant conducted such a study and submitted it to DPR (Schaefer, 2002).

With the submission of the new 6-week subchronic dog study, DPR reevaluated the database to determine the appropriate endpoint and critical NOEL to characterize the risk from subchronic inhalation exposure to methyl bromide. The 1999 draft RCD has been revised and the final document (DPR, 2002a) is pending public release. The 2002 RCD contains essentially the same toxicology and hazard identification sections as in the 1999 draft RCD but does not include the Schaefer (2002) study.

II. Subchronic Inhalation Toxicity of Methyl Bromide

II.A. Overview of Database

Descriptive summaries of all subchronic toxicity studies by inhalation and oral routes are presented in the completed Risk Characterization Documents (DPR, 1999 and 2002a). In summary, subchronic inhalation exposure of laboratory animals to methyl bromide resulted in altered brain catecholamine levels, decreased brain tyrosine hydroxylase activity, neurotoxicity, tissue degeneration (brain, nasal cavity, heart, testes, adrenal glands, thymus, spleen, and

³ Critical NOEL is the NOEL selected for the calculation of margin of exposure (MOE= NOEL/exposure). It is based upon on the most sensitive endpoint (effect) and its use addresses all other effects observed at higher doses.

⁴ The National Toxicology Program defines equivocal as “marginal evidence, which may be chemical related.”

kidneys), and death. Based on overt signs of neurotoxicity, the dog, rabbit, and monkey were more sensitive to methyl bromide than other species (rat, mouse, and guinea pig). The primary finding after oral exposure by gavage in the rat was hyperplasia of the forestomach. A decrease in body weight gain and food consumption was observed in rats given microencapsulated methyl bromide mixed in the feed.

II.B. Specific Studies on the Neurotoxicity of Methyl Bromide

For subchronic inhalation toxicity, the primary effect was neurotoxicity, which included neurobehavioral changes and effects in the brain (reduced brain weight and lesions). Studies pertinent for this document are the two dog studies (Newton, 1994a; Schaefer, 2002), and three rat studies (Eustis *et al.*, 1988; NTP, 1992 and Eustis, 1992; and Norris, *et al.*, 1993 a and b).

Newton, 1994a

The Newton study (1994a) was a pilot study conducted to determine the doses for the SB 950 required chronic toxicity study in dogs. The registrant used the results to show that the existing toxicology database was adequate to characterize methyl bromide neurotoxicity. DPR concurred and waived the requirement for the chronic inhalation toxicity study in dogs.

Beagle dogs (4 dogs/sex/group) were exposed to methyl bromide (100% pure; 0, 5, 11/158, 26, 53, 103 ppm as measured concentrations) by whole body inhalation for 7 hours per day, 5 days per week (Newton, 1994a). The original exposure duration was 4 weeks (23-24 exposures) and this was carried out for the 0 (2/sex), 5, 10, 26, 53, and 103 ppm groups. After the 4-week period, the exposures of the two lowest groups, 5 and 11 ppm, were modified. Since no effects were observed in the 5-ppm group after that duration, the exposure for this group was extended for another 2 weeks (30 exposures) along with the rest of the controls (2/sex). After 24 exposure days, the dose for the 11-ppm group was increased to 158 ppm.

Serum bromide levels increased with the dose at ≥ 26 ppm. Body weight loss (25%) and neurotoxicity were seen in the dogs exposed to 158 ppm. These dogs showed decreased activity on the second exposure day (the first dose was on a Friday and the second dose was on the following Monday). They were reported in poor condition during the final (6th) exposure (Table 1). The next day, three 158-ppm males had to be sacrificed due to severe toxicity (opisthotonos, irregular gait, opening and closing of the jaws and convulsions). The remaining 158-ppm dogs exhibited: nystagmus, intention tremors, ataxia, irregular gait, marked depression and inability (unwillingness) to stand and perform postural response. Elevated levels of protein and bilirubin were measured in the urine of the 158-ppm dogs. Histological examinations showed that each of the 158-ppm dogs had cerebellar lesions (vacuoles in the granular layer) and olfactory degeneration; the males also showed adrenal cortex lesions (zona fasciculata, cytoplasmic vacuoles).

Decreases in body weight gain and less severe neurotoxicity (tremors, emesis, decreased activity during exposure but not post-exposure) were seen in the 103-ppm dogs. The loss in body weight was statistically ($p < 0.05$) significant for all weekly measurements for males and from week 2 on for females. A decrease in activity was noted starting on exposure day 9 involving

most or all of the animals. Tremors were observed in 1 of 8 dogs on day 10. Emesis was observed in 1 dog on days 9 and 10. One 103-ppm male exhibited a cerebellar lesion similar to that seen in the 158-ppm dogs. In the 53-ppm group, a decrease in activity during exposures also was noted (in 2 dogs), starting on exposure day 14. However, no abnormal findings were reported for the 53-ppm group in post-exposure examinations. The NOAEL for 23-24 exposure days was 53 ppm for neurotoxicity at 103 ppm and 158 ppm.

The female dogs which were exposed the longest to methyl bromide (5 ppm group) had reduced absolute spleen weights (55% of control compared to 75% of control for the male group) and two 5 ppm females were observed by an animal neurologist at the end of test week 6 to be less responsive than expected. The neurologist noted that one dog was “quite unresponsive and motionless” when given the opportunity to move freely. A second dog was noted to have “stood quietly, appeared depressed, while lifting its left forelimb.” There were no reflex or postural deficits noted for these dogs. In addition to the 6th week exam, the neurologist had examined all dogs in the study on two other occasions: pre-test and at the end of week 4. He had reported that the dogs were suitable for testing (pre-test exam) and that no neurological effects were observed after 4-weeks of exposure. From exposure days 31 to 34 (after the week 6 exam to the end of experiment), the report noted no abnormal activities in these dogs. The neurologist did not examine them at this time. The LOAEL for 34 exposure days was 5 ppm for decreased spleen weight (females) and decreased responsiveness (females). The U.S. EPA also established 5 ppm as the LOAEL based in the decreased responsiveness observed after 7 weeks of exposure (Chin, 2001)

Table 1. The neurotoxicity of methyl bromide in dogs in a 4-6 week toxicity study.^a

Concentrations mean ± sd (ppm)	Onset	Clinical Signs and Incidences^c	Clinical Signs with Additional Exposure
158± 7 ^b	Day 2	Decreased activity (8/8)	Severe neurotoxicity, cerebellar lesions (8/8)
103± 9	Day 9	Decreased activity (3/8)	Day 9 to 10: emesis (1/8), tremor (1/8), decreased activity (3/8); week 5: cerebellar lesions (1/8)
53± 4	Day 14	Decreased activity (2/8)	
26± 1	23-24 exposures	No effects observed	
5± 0.4	30 exposures	Decreased responsiveness (2/8)	

^{a/} Data from Newton, 1994a.

^{b/} The dogs were exposed to 11 ppm for 24 exposure days, then 158 ppm for 6 exposure days.

^{c/} Incidences as number of dogs affected/total are shown in parentheses.

Schaefer, 2002

Beagle dogs (4/sex/group) were exposed to methyl bromide (100% pure, nominal concentrations of 0, 5, 10, or 20 ppm) by whole body inhalation for 7 hours per day, generally 5 days per week (exceptions: ≤ 3 days in initial week; 6 days in penultimate week; ≤ 4 days in final week) for at least 30 exposure days (Schaefer, 2002). During early part of the study, the chamber concentration was not well controlled due to technical problems (more details of the problem are in DPR, 2002b). During study weeks -2, -1, 2, 4 and 6, Functional Observation Battery (FOB) testing was performed, followed by motor-activity measurements using an automated apparatus. By experimental design, the cumulative number of exposure hours on a weekly basis before the FOB and motor-activity testing were conducted in study week 2, 4 and 6 may have ranged from 14 hours to 35 hours within each group. If the cumulative number of hours of exposure in the week of the FOB-motor activity testing is important for observing neurological effects in such testing, this design may not have been optimal. After exsanguination, *in situ* perfusion with paraformaldehyde and glutaraldehyde was performed to allow for the histological examination of nervous-system tissues in accordance with U.S. EPA neurotoxicity guidelines.

No deaths occurred during the study and observed effects were considered mild in comparison to the worst effects reported in Newton (1994a). There were no treatment-related effects on the following: body weight, feed consumption, rectal temperature, and fixed spleen weight (absolute or relative to body weight). Necropsy also showed no treatment-related effects.

In the FOB testing, absence of the proprioceptive-placing response during table top measurements were noted in two consecutive sessions with a male from the 10 ppm group, in three consecutive sessions with a male from the 20 ppm group, and in the first testing session with a female from the 20 ppm group (Table 2). Pretesting data and historical negative-control data indicate that it is rare for untreated dogs not to exhibit this response, especially in a repetitive manner. In the motor activity testing, there was no obvious treatment effect but supplemental information is needed to complete the evaluation of these data. Clinical-examination findings included the following: emesis by two 20 ppm females at the end of study week 4; clear discharge from eye(s) by two 20 ppm males (almost daily) and, possibly, a 20 ppm females at the end of the study week 4; and feces-related findings (soft feces, mucoid feces, mucoid feces with blood, and/or diarrhea) in four 20 ppm males, two 20 ppm females, two 10 ppm males and one 10 ppm female. Clear eye discharges and feces-related findings were also observed in other animals (including the control). However, as shown in Table 2, there were increased total incidences and number of animals involved for these two effects in males exposed to increasing methyl bromide concentration. For the females, such a relationship was evident only for the feces-related effects, but not for eye discharges.

One 5-ppm male exhibited twitching (three times over four hours on one day) and tremors (on consecutive days in the final test week) (Table 2). Also three 5 ppm males that had not been observed to vomit during the pretest period did so (once each) while on test; in one case, emesis occurred after the first or second day of exposure, when there may have been difficulties in controlling methyl bromide release into the inhalation chamber. Although the researchers considered the animal with twitching and tremors to be afflicted with idiopathic febrile necrotizing arteritis, the basis for this diagnosis was not well documented and

supplemental information is needed. The data reviewer for the Medical Toxicology Branch established 5 ppm as the LOAEL for the study based on twitching and tremors, and emesis at 5 ppm. Major deficiencies include: (1) lack of positive-control data regarding the FOB testing, motor-activity measurements and nervous-system histology were either inadequate or not provided; (2) the histological evaluation of the nervous-system tissues did not include the use of special stains and it is unclear whether the findings from the previous dog inhalation study (Newton, 1994a) were used to guide the histological examination in this study; and (3) some methods, data and a protocol deviation regarding the male presumed to be exhibiting idiopathic febrile necrotizing arteritis were not provided.

Table 2. Effects of methyl bromide in dogs in a 6-week toxicity study.^a

Effects	Methyl bromide concentration (ppm)							
	Males				Females			
	0	5	10	20	0	5	10	20
Tremor	0/4	1/4 (#8738)	0/4	0/4	0/4	0/4	0/4	0/4
Twitching	0/4	1/4 (#8738)	0/4	0/4	0/4	0/4	0/4	0/4
Emesis	0/4	3/4 (#8721, #8724, #8728)	0/4	0/4	0/4	0/4	0/4	2/4 (#8752, #8759)
Lack of proprioceptive placing								
-2 week	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
-1 week	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
week 2	0/4	0/4	1/4(#8732)	1/4(#8723)	0/4	0/4	0/4	1/4(#8751)
week 4	0/4	0/4	1/4(#8732)	1/4(#8723)	0/4	0/4	0/4	0/4
week 6	0/4	0/4	0/4	1/4(#8723)	0/4	0/4	0/4	0/4
Clear discharge from eyes	0/4 (0)	1/4 (2)	0/4 (0)	3/4 (75)	2/4 (57)	1/4 (29)	2/4 (3)	3/4 (14)
Feces-related effects ^b	2/4 (7)	3/4 (8)	4/4 (22)	4/4 (46)	2/4 (8)	2/4 (6)	1/4 (8)	3/4 (16)

^{a/} Data from Schaefer, 2002. Incidence = number of dogs affected/number examined. for tremor, twitching, and proprioceptive placing, the dog identification number (#) is noted in the parenthesis. For eye discharge and feces-related effects, the total incidence for each group is noted in the parenthesis.

^{b/} Feces-related effects included: soft feces, mucoid feces, mucoid feces with blood, and/or diarrhea.

In comparison, the authors of the study established a NOEL of 20 ppm with no effects at the highest dose tested. The tremors and twitching observed at 5 ppm were dismissed as treatment-related because the affected dog was “diagnosed” to have febrile necrotizing arteritis and there were lack of changes in the FOB parameters, motor activity, and neuropathology. The absence of proprioceptive placing response at 10 ppm and 20 ppm was also dismissed because there were no other corroborative FOB findings or histological changes.

The registrant had sent this study to Dr. J. Chambers, a member of the NRC committee who reviewed the draft inhalation RCD. Dr. Chambers agreed with the authors on the findings and that the NOEL should be at 20 ppm. She considered the study well organized, well designed, and carefully conducted; no deficiencies were mentioned in her review. Furthermore, she recommended that the Schaefer study should take precedence over the Newton study in the selection of the critical NOEL.

For another internal review of this study, DPR submitted the study to Dr. T. Kellner, a staff with the Medical Toxicology Branch with expertise on FOB studies and neurotoxic responses (Kellner, 2002). Dr. Kellner noted that FOB is a type of neurotoxicity screening battery and its use depends on whether it is for the identification of a new neurotoxicant or for delineating a neurological threshold for risk assessment of a known neurotoxicant. For the latter, FOB in isolation or in relatively few clusters may be important for use in risk assessment but should be considered in terms of dose-response relationship and biological plausibility for such an effect based on known mode of toxicity. Dr. Kellner’s review focused on the proprioceptive placing response. Since there were no complementary reflex findings, no microscopic changes, and a lack of dose-response, his overall impression was that the proprioceptive placing changes were incidental findings. He did not address the other findings or the selection of a NOEL for the study.

DPR also sent this study to Dr. K. Pinkerton, University of California at Davis, for an external review (Pinkerton, 2002). Dr. Pinkerton also recommended the Schaefer study to set health safety standard for methyl bromide inhalation exposure. He cited the Schaefer study as a more complete study whereas the Newton study was a range-finding study, not designed to test neurotoxicity. Dr. Pinkerton considered the lack of proprioceptive placing a treatment-related effect because it was not observed at pretest and no control animals were affected during study. Dr. Pinkerton suggested that the NOAEL should be at 5 or 10 ppm rather than 20 ppm. He did not consider anatomical changes being necessary for an effect to be identified as an indication of neurotoxicity.

After the above reviews, the senior scientific staff of the Medical Toxicology Branch also evaluated this study. The consensus of the senior staff was that the 5-ppm dose should be the No-Observed-Adverse-Effect Level (NOAEL), instead of LOAEL, for the study (Patterson, 2002). This consensus was, however, not unanimous; there was a minority opinion that 5 ppm should be the LOAEL. All staff agreed that the proprioceptive placing response found at 10 and 20-ppm groups was a treatment-related effect. The determination of the 5-ppm dose as a NOEL was primarily based on the lack of dose-response for the finding of twitching and tremors. There was only one dog affected at 5 ppm but none at the 10-ppm and 20-ppm groups. In establishing the 5 ppm as the NOEL, the staff recognized that there were uncertainties to this determination. Some

were already discussed by the Medical Toxicology Data Reviewer in the previous paragraphs. Additional uncertainties included: the difficulty for demonstrating a dose-response when the selected doses were close (just 4-fold difference from the low dose to the high dose) and lack of pretest individual data for clinical signs. Questions and issues raised by the reviewers are summarized in Table 3.

Table 3. Questions and issues related to the effects observed in the 6-week toxicity study with dogs.^a

1. Are effects observed at 5 ppm treatment-related?		
Effects	Yes, the effects are treatment related because:	No, the effects were not treatment related because:
Twitching (3 times over four hours on one day) and tremors (consecutive days in the final test week)	<ul style="list-style-type: none"> • The effect was observed more than once • The effect consistent with methyl bromide neurotoxicity • There are only 8 dogs in the group so 1/8 can be important and may be related to individual sensitivity 	<ul style="list-style-type: none"> • Only 1 dog affected. • The dog was afflicted with febrile necrotizing arteritis.
Emesis in 3 males	<ul style="list-style-type: none"> • These animals did not vomit during pre-test. • Two 20-ppm females also vomited once. 	Emesis occurred only once per dog
2. Is there sufficient evidence to show that the 5-ppm dog with twitching/tremor was afflicted with febrile necrotizing arteritis?		
Febrile necrotizing arteritis syndrome:	Yes, there is sufficient evidence because:	No, the evidence is insufficient because:
Intermittent fever	The dog showed 104.1°F once on study week 4.	The temperature increase was similar to maximum value (103.5°F and 103.3°F) in control and other treated groups.
Lethargy	The dog showed hypoactivity (once during 1-hr postexposure).	Hypoactivity was observed at a different time from when temperature increased to 104°F.
Stiffness in the hindlimb gait and neck	(not observed)	There was no evidence for this effect. All FOB testing was normal.
Elevated WBC count	(no hematology data)	No hematology data were reported.
Periarteritis	Periarteritis was observed for this dog.	No histology reported for others dog (control or other treated dogs) to confirm that only one dog was affected.

Table 3. Questions and issues related to the effects observed in the 6-week toxicity study with dogs (continued).^a

3. Is the absence of proprioceptive-placing response at 10 ppm and 20 ppm a treatment-related effect?		
Effect	Yes, the effect is treatment-related because:	No, the effect is not treatment-related because:
Lack of proprioceptive placing response at 10 ppm and 20 ppm	<ul style="list-style-type: none"> • Effect observed in two doses and increased incidences. • Effect was not observed during pretest week -2 with more animals (20 dogs/sex), tested twice each; nor at study week -1 with 16 dogs/sex for this study. Control dogs in conducting laboratory showed low incidence for this lack of response. • Proprioceptive placing is a sensory function which may not be detected by motor-strength tests. • Doses were low and only 4-fold difference between the high and low dose. Dramatic neurological effects were not expected. • Histological/anatomical changes are not necessary to support finding. • For known neurotoxicant, effects in isolation or in clusters are pertinent for risk assessment. 	<ul style="list-style-type: none"> • No corroboratory evidence of weakness from wheel-barrowing or hemistanding/hemiwalking tests • No other signs of neurotoxicity • No histological changes.

^{a/} Data from Schaefer, 2002, worksheet for the study (DPR, 2002b), Kellner (2002), Pinkerton (2002), Chambers (2002), and Patterson (2002).

Eustis *et al* (1988)

Fischer-344 rats and B6C3F1 mice were exposed to methyl bromide (99.8% pure, nominal concentration of 160 ppm) for 6 hours per day, 5 days per week (Eustis *et al.*, 1988). The experiment was intended for 6 weeks but was terminated after 2 weeks for the male rats and mice (both genders) because of high mortality (more than 50% death). At the end of the 30 exposures, the survival of the female rats was 50%. There were no treatment related findings in the parameters measured in the clinical chemistry or urinalysis. The treated rats showed curling and crossing of hindlimbs, forelimb twitching, and tremors. There were significant ($p < 0.05$) decreases in the body weight (68% of control), and the weights of lungs, heart, spleen, right kidney, brain (Table 4), liver, and right testes of the males. For the females, significant ($p < 0.05$) decreases in body weight (82% of control), and the weights of lungs, right kidney, brain (Table 4), and liver were observed. Pathological lesions were found in the brain (necrosis and loss of neurons in the cerebral cortex, hippocampus, and thalamus), testes (degeneration and atrophy of the seminiferous tubules), nasal olfactory epithelium (necrosis and degeneration), heart (cardiomyopathy), adrenal cortex (cytoplasmic vacuolation), liver (hepatocellular necrosis), thymus (atrophy), and spleen (lymphoid depletion).

In this study, the methyl bromide-treated mice showed red urine, lethargy, and neurological effects (curling and crossing of the hindlimbs, forelimb twitching and tremors). Treated male and female body weights at termination were only 74% and 82% of the control values ($p < 0.001$, both sexes), respectively. Absolute organ weights which were significantly ($p < 0.05$) lower in both sexes (unless noted) included: lungs, heart, spleen (males), right kidneys (males), thymus, brain (Table 4), and liver. Histological examination of the tissues showed lesions in the brain (necrosis and loss of neurons in the cerebellum and cerebral cortex), kidney (nephrosis, dilatation and increased cytoplasmic basophilia), testes (degeneration), nasal cavity (degeneration and atrophy, males), heart (cardiomyopathy), adrenal gland (atrophy of the inner-zone of the adrenal cortex, females), thymus (atrophy), and spleen (lymphoid depletion and hematopoiesis).

Table 4. Effect of methyl bromide on the brain of rodents exposed for 2-6 weeks.

Effects ^a	Male		Female	
	0	160 ppm	0	160 ppm
Rat				
Duration of exposure	14 days		30 days	
Number of samples	10	5	10	5
Body weight (g)	164.2	112.0*** (68%)	150.5	123.6*** (82%)
Brain weight (g)	1.66	1.56* (94%)	1.68	1.52*** (90%)
Brain lesions				
Cerebral cortex- neuronal necrosis	0/10	5/10	0/10	10/10
Cerebral cortex – gliosis	0/10	0/10	0/10	1/10
Hippocampus- neuronal necrosis	0/10	1/10	0/10	2/10
Hippocampus- gliosis	0/10	0/10	0/10	1/10
Thalamus- neuronal necrosis	0/10	2/10	0/10	4/10
Thalamus-gliosis	0/10	0/10	0/10	1/10
Cerebellum- mineralization	0/10	0/10	0/10	2/10
Mouse				
Duration of exposure	10 days		8 days	
Number of samples	20	4	20	10
Body weight (g)	27.3	20.1*** (74%)	20.2	16.53*** (82%)
Brain weight (g)	0.45	0.43* (96%)	0.45	0.42*** (93%)
Brain lesions				
Cerebral cortex – neuronal necrosis	0/20	11/20	0/20	0/20
Cerebellum- neuronal necrosis	0/20	12/20	0/20	10/20
Hemorrhage	0/20	1/20	0/20	0/20

a/ Data from Eustis *et al.*, 1988. *, **, *** significantly different from control at p<0.05, p<0.01, p<0.001. Values in parenthesis are % of control. Body weight and brain weight were mean values.

NTP (1992) and Eustis (1992)

In a longer-term study, Fischer-344 rats were exposed to methyl bromide (99.8% pure, nominal concentrations of 0, 30, 60, or 120 ppm) for 6 hours per day, 5 days per week for 13 weeks (NTP, 1992; Eustis, 1992). No mortality was observed. The body weights were decreased as early as week 6 of exposure. By 13 weeks, the body weights of the 60 and 120-ppm males were 90% and 84% of control, respectively. The brain weights of the 120-ppm groups were significantly ($p<0.01$) decreased to 92% (males) and 93% (females) of control values (Table 5). There were occasional neurobehavioral effects (such as decreased startle response amplitude, increased startle response latency, and decreased grip strength) in the 120-ppm groups, which were significantly ($p<0.05$) different from control values; however, they were not related to exposure duration. Significant ($p<0.05$) decreases in the mean hematocrit, hemoglobin, and erythrocyte counts were detected in the 120-ppm females only. A significant decrease in the erythrocyte count also was noted in the 60-ppm females. An increase in the incidences of olfactory epithelial dysplasia and cysts (irregularity in mucosal thickness and focal cavity spaces) was found in both sexes of the 120-ppm groups. The NOEL was 60 ppm for neurotoxicity and olfactory epithelial dysplasia and statistically significant decrease in brain weight at 120 ppm.

When B6C3F1 mice were exposed to methyl bromide (99.8% pure; nominal concentrations of 0, 10, 20, 40, 80, or 120 ppm) for 6 hours per day, 5 days per week for 13 weeks, the survival rates were 83% for the 120-ppm males and 100% for the females (NTP, 1992; Eustis, 1992). In the 120-ppm group, body weight gain was 58% of control ($p<0.05$, males), and brain weights were 92 to 94% of control ($p<0.01$, both sexes) (Table 5). Clinical signs (severe curling, crossing of the hindlimbs, and twitching of the forelimbs) were observed in the 120-ppm groups, with greater severity occurring in the males than in the females. Alterations in a few of the neurobehavioral responses were observed, primarily in the 80-ppm groups; no data were reported for the 120-ppm groups. Significant ($p<0.05$) changes in the hematological parameters, which were dose-related included: decreased mean cell hemoglobin (40 to 120 ppm males, 120 ppm females), decreased mean cell volume (40 to 120 ppm males), increased erythrocyte count (40 to 120 ppm males), and increased hemoglobin (120 ppm males). No compound-related lesions were observed by histological examination. The NOEL was 20 ppm for changes in blood parameters at 40 ppm and neurobehavioral responses at 80 ppm.

Table 5. Effect of methyl bromide on brain weights of rodents exposed for 13 weeks.^a

Effect	Male						Female					
Rat												
Concentration	0	30	60	120 ppm	0	30	60	120 ppm	0	30	60	120 ppm
No. samples	10	10	9	10	10	10	10	10	10	10	10	10
Brain weight (g)	1.90	1.87	1.83	1.74** (92%)	1.77	1.75	1.75	1.65** (93%)				
Mouse												
Concentration	0	10	20	40	80	120	0	10	20	40	80	120
No. samples	10	10	10	10	8	10	10	10	10	10	8	10
Brain weight (g)	0.46	0.47	0.46	0.46	0.45	0.43** (93%)	0.48	0.47	0.49	0.47	0.48	0.44** (92%)

^{a/} Data from NTP, 1992 and Eustis, 1992. ** denotes significance at $p<0.01$.

Norris *et al.*, 1993 a and b

CD rats (15/sex/group) were exposed to methyl bromide (>99% pure; nominal concentrations of 0, 30, 70, or 140 ppm) by inhalation for 6 hours per day, 5 days per week for 90 days (Norris *et al.*, 1993 a and b). Neurobehavioral testing was done pre-exposure and at the end of study weeks 4, 8 and 13. Testing included automated assessments of motor activity and a Functional Observation Battery (FOB). Two 140-ppm males died on test (days 12 and 27); the latter had convulsions and tremors before dying. One other 140-ppm male that survived until the end of the study also exhibited clonic convulsions and tremors. The 140 ppm groups (both sexes) weighed significantly less than the controls, starting at study week 4 while the 70-ppm female group exhibited a body weight reduction, starting about study week 9. The mean body weights were 87% of control values on week 13 for 140-ppm groups, and 92% for the 70-ppm females. Female groups exposed to methyl bromide exhibited a dose response for reduced absolute brain weight (Table 6). The reductions were statistically significant ($p \leq 0.01$) for all dose levels and were 96%, 95%, and 90% of control for 30 ppm, 70 ppm, and 140 ppm, respectively. The report considered the effects at 30 and 70 ppm as slight but stated that a relationship of this finding to methyl bromide exposure could not be ruled out.

FOB testing identified effects only in the 140 ppm groups; some effects were evident as early as study week 4 (Table 6). Findings included: ataxia (5 females, 1 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 activity; both were evident as incipient effects in study week 8.

Histological findings included: brain lesions at multiple sites (four 140 ppm males; neuronal loss, neuronal necrosis, malacia); peripheral nerve degeneration and/or vacuolation (140 ppm, 2/sex affected; one 30 ppm female affected); and olfactory epithelium dysplasia (140 ppm, 3/sex affected). The NOAEL was < 30 ppm based on reduced brain weight at the lowest dose tested. Neurobehavioral testing effects were observed at ≥ 70 ppm. Submission of “validation” training and positive control data (Gill, 1989 a, b, c, and d) generated 5 years before the methyl bromide testing using different personnel than those in the methyl bromide testing (Driscoll *et al.*, 1994) were considered inadequate. Therefore, this study was considered unacceptable and not upgradeable by DPR because of the lack of FOB validation.

Table 6. The neurotoxicity of methyl bromide in rats exposed for 13 weeks.^a

Effects	Males				Females			
	0	30	70	140	0	30	70	140
Body weight	500.2	512.1	491.9	430.2**	290.8	281.0	268.8*	251.7**
Brain weight (g)	2.301	2.346 (102%)	2.285 (99%)	2.154** (94%)	2.146	2.057** (96%)	2.038** (95%)	1.934** (90%)
FOB- Ataxic gait								
4 week	0/10	0/10	0/10	0/10	0/10	0/10	0/10	3/9
8 week	0/10	0/10	0/10	0/10	0/10	0/10	0/10	2/9
13 week	0/10	0/10	0/10	1/10	0/10	0/10	0/10	1/9
Arousal								
4 week	0/10	0/10	1/10	1/10	0/10	0/10	0/10	0/9
8 week	3/10	5/10	7/10	4/10	0/10	0/10	0/10	4/9
13 week	5/10	9/10	9/10	5/10	1/10	0/10	1/10	5/9
Rears								
4 week	8.9	9.9	8.4	7.1	15.8	15.2	15.5	10.6*
8 week	4.6	4.3	2.4	2.9	14.8	15.9	13.7	6.6*
13 week	2.5	0.7	1.1	1.3	14.7	11.4	11.0	4.9**
Righting								
4 week	2/10	6/10	1/10	1/10	1/10	1/10	1/10	0/9
8 week	5/10	3/10	3/10	6/10	1/10	1/10	0/10	3/9
13 week	4/10	4/10	4/10	8/10	0/10	2/10	0/10	2/9
Hind leg splay								
4 week	7.3	8.2	8.3	10.1**	9.7	7.6**	8.5	9.4
8 week	8.2	8.1	8.7	10.0	8.2	7.3	8.2	7.7
13 week	6.9	7.7	7.8	10.2**	7.4	6.7	7.6	7.5
Motor activity								
4 week	1350	1614	1590	1509	1721	1828	1677	1460
8 week	1551	1787	1500	1495	1684	1756	1353	1411
13 week	1501	1402	1508	1438	1517	1627	1099**	1159**
Histological findings								
Brain-neuronal loss, necrosis, and/or malacia	0/6	0/6	0/6	4/8**	0/6	0/6	0/6	0/6
Peripheral nerve-degeneration and/or vacuolation	0/6	0/6	0/6	2/6	0/6	1/6	0/6	2/6
Olfactory epithelium-dysplasia	0/6	0/6	0/6	3/8	0/6	0/6	0/6	3/6

^{a/} Data from Norris *et al.* (1993 a and b). n=10, ** significantly different from control group (p<0.01). Arousal=number inactive but alert/total examined, Righting=number of abnormal righting/total examined, Rears=events (mean value), hind leg splay= in centimeters. Motor activity or rearing=mean cumulative test session counts.

III. Hazard Identification

The most appropriate data for the hazard identification are those from human studies. However, human case reports of methyl bromide (as cited in DPR, 1999 and 2002a) did not provide sufficient data for evaluation. In the absence of human data, results from animal studies were extrapolated to humans assuming that the effects observed in laboratory animals would also be observed in humans. This extrapolation was appropriate especially for neurotoxicity, an effect reported in both human and experimental animals exposed to methyl bromide.

III.A. Comparison of Study Design

A comparison of the studies in the database showed that the two dog studies were pivotal in the selection of the critical NOEL and endpoint. The dog remained the most sensitive species for the neurotoxicity of methyl bromide based on previous comparison of the toxicity and the NOEL for the new study (Schaefer, 2002) established at either <5 ppm or 5 ppm. This discussion explored various aspects of the study design between the 2 dog studies that might impact on the interpretation of the result.

1. Purpose of study

The main purpose of both studies was the same: to evaluate the neurotoxicity of methyl bromide in dogs. They differed in design in that the Newton study (1994a) was designed as a range-finding study to determine the appropriate concentrations for chronic toxicity testing while the Schaefer study (2002) was conducted to confirm/refute the results of the Newton study. Both studies were conducted under Good Laboratory Practices.

2. Number of animals used

With regard to specific experimental protocol, both studies used 4 dogs/sex/group. The NRC considered this number of animals too few replications. The FIFRA guidelines for subchronic toxicity studies indicated that ≥ 10 animals/sex should be used (OPPTS 870.6200-Neurotoxicity Screening Battery-August, 1998). While the guidelines focused on the use of rats, they also acknowledge the potential use of dogs. Most dog studies are conducted using 4 dogs/dose/gender. Statistical comparison is problematic with only four-animals/sex/test group, especially when low doses are used as in the case with methyl bromide and dramatic effects are not expected.

3. Exposure concentration

In the Newton study, the lowest dose was 5 ppm and the highest dose was 158 ppm. There were no technical difficulties reported. In the Schaefer study, the dose range was narrow and ranged from 5 ppm to 20 ppm. There were difficulties in controlling the methyl bromide concentration released into the chamber during the early part of the Schaefer study. Some animals in all groups might have been exposed to high concentrations for a short time. It is unknown how this technical problem affected the outcome of study.

4. Duration of exposure

In the Newton study, only the lowest dose (5 ppm) was exposed for 6 weeks while the other groups were exposed for 4 weeks. The duration for the 5-ppm group, as well as half of the animal from the control group, was extended because no effects were observed after 4 weeks. Since this was a range-finding study, a change in protocol in search of an effective dose seemed reasonable. In the Schaefer study, all treated groups were exposed to methyl bromide for 6 weeks. In both studies, the dogs were exposed for 7 hours per day.

5. Neurotoxicity evaluation

In the Newton study, the observations consisted of cage-side observations and evaluation by a veterinary neurologist. In the Schaefer study, the neurotoxicity evaluation also included FOB and motor-activity testing. Both studies examined the brain tissues.

One problem with the Schaefer study evaluation, with respect to hazard identification, was the lack of a positive control and a methyl bromide effective dose for the FOB and motor activity testing. If the finding on proprioceptive placing and other effects are dismissed, one question is whether the FOB assays were sensitive enough to detect methyl bromide neurotoxicity in dogs. This is particularly important since the FOB study in dogs is uncommon; neither the DPR database nor an open literature search showed any other such studies. With methyl bromide, the FOB evaluation in rats showed that other endpoints were more sensitive than those used in the FOB. For example, the LOEL was 30 ppm for decreased brain weight while the LOEL was 70 ppm for changes in FOB and decreased in motor activity (Norris *et al.*, 1993 a and b). FOB tests with rats are commonly used in the screening for neurotoxicity after exposure to organophosphate pesticides.

Conclusion: Considering the various aspect of study design, more weight should be placed on the Schaefer study, compared to the Newton Study, because of the more extensive neurotoxicity evaluation. However, the results from the Newton study deserved some weight in the overall evaluation because there was no evidence that the protocols used in the Newton study resulted in unreliable or false positive results. The finding of treatment-related effects showed that the dogs were at least adequately evaluated.

III.B. Comparison of Endpoints and NOELs

With both dog toxicity studies, several endpoints could be considered in the hazard identification. Since there were few animals used in these studies, any effects observed in only one or few animals could be important in the overall evaluation of the study as they might reflected greater sensitivity in the particular individual versus other animals in the group. Additionally, a clear dose-response might not be evident for some endpoints observed at the lower dose range. As stated in the U.S. EPA neurotoxicity risk assessment guidelines, mild and inconsistent effects are not unexpected when the exposure is at or near the NOEL (U.S. EPA, 1998).

1. Newton Study (1994a)

In the Newton study, the key endpoint was decreased responsiveness in two female dogs observed at the lowest dose (5 ppm) at the end of 6-week exposure (Table 1).

- This endpoint was significant because reduced responsiveness was observed only in the treated dogs and was not considered normal behavior. The study report stated that the normal response for the dogs would have been excitement when the chambers were approached for observation. This effect was consistent with neurotoxic characteristics of methyl bromide.
- A dose-response relationship was not obvious for reduced responsiveness/decreased activity in this study because there was no effect observed at one of the doses (26 ppm; Table 1). It is unknown if the dogs were truly not affected or that the experiment was terminated too soon (at 4 weeks instead of 6 weeks). However, if the result at 5 ppm was compared with the other three concentrations in the study, a dose-response relationship could be established to show that decreased activity was observed sooner at higher concentrations than at lower concentrations (Table 1). At 5 ppm, a decreased responsiveness was not observed until after 6 weeks of exposure. At higher concentrations, there was evidence that this effect was observed with fewer exposures and that it was an early sign of neurotoxicity. At 53 ppm, decreased activity in 2/8 dogs was first observed on day 14. When the concentration was increased to 103 ppm, an additional dog (3/8) showed decreased activity and at an earlier time (on day 9). When the concentration was at 158 ppm, decreased activity was observed with only two days of exposures in all 8 dogs in the study. In both 103 ppm and 158-ppm groups, the neurotoxicity progressed from decreased activity during the early part of the study to severe neurotoxicity with clinical signs including tremors and convulsion, and brain lesions with additional exposures.
- The lowest dose, 5 ppm, was the LOEL for the study. The U.S. EPA also established 5 ppm as the LOEL based on the same findings as DPR.

b. Schaefer Study (2002)

- Two of the endpoints were tremor and twitching observed in one dog at 5 ppm, the lowest dose tested (Table 2). While these effects were consistent with the neurotoxicity of methyl bromide, there was no dose-response relationship since they were not observed in any other

treated groups. The author of the report and two reviewers dismissed these findings because this dog was diagnosed to have idiopathic febrile necrotizing arteritis. However, as noted by the Medical Toxicology Data Reviewer, the basis for the diagnosis and the relationship between this disease and these effects was not clear (Table 3).

- Another endpoint was the absence of proprioceptive placing response found at 10-ppm and 20-ppm dogs (Table 2). A dose-response relationship was evident as there was no effect at 5 ppm; 1 animal affected in two testings at 10 ppm, and two animals affected in 4 testings at 20 ppm (Table 2). Since the dose interval was narrow, only a 4-fold difference between the high and low doses, the dose-response relationship was relatively shallow. The 5-ppm dose could be the NOEL for this endpoint as indicated by Patterson (2002) and Pinkerton (2002). On the other hand, some reviewers did not consider this endpoint as significant because there were no corroborative effects from the FOB or motor activity testing, or histological change (Kellner, 2002; Schaefer, 2002).
- Other endpoints identified included emesis, clear eye discharges, and feces-related effects (Table 2). There was no dose-response relationship for emesis as only the 5-ppm males and 20-ppm females were affected. With clear eye discharges and feces-related effects, there were increased incidences with increasing concentration (except for the 20 ppm females and eye discharges).
- When all the identified effects were considered as a spectrum of effects, there appeared to be a dose-response relationship with tremor/twitching observed at the lowest dose and other effects involving more animals and increased incidences at the higher doses. In addition, two of the males dogs (one in the 10 ppm and one in the 20 ppm group) that lacked the proprioceptive-placing response) also had mucoid feces or feces containing blood.
- Because of the varied interpretations on findings as individual endpoint and as a spectrum of effects, several NOAELs for the study had been identified and included: <5 ppm (Medical Toxicology Data Reviewer), 5 ppm (Patterson, 2002; Pinkerton, 2002), 10 ppm (Pinkerton, 2002), and 20 ppm (Schaefer, 2002; Chambers, 2002).

Conclusion: Both dog studies showed effects that could be attributed to methyl bromide treatment. The dose-response relationship was more evident for some effects but not others due to limitations in the study design problems (exposure duration, few animals tested, narrow dose range). The decreased activity or responsiveness in the Newton study suggested a dose and time of onset relationship for this endpoint, except with one dose where the experiment might have terminated too early for the effect to be manifested. With the Schaefer study, a dose-response relationship was more evident for the absence of proprioceptive placing response than for other effects considered as individual endpoints or as a spectrum of effects.

III.C. Determination of the Critical NOEL

The determination of the critical NOEL is a weight of evidence process considering all studies in the database with respect to the quality of the study, the relevancy of the effect, and the magnitude of the NOEL. As discussed in the previous section, the quality of the Schaefer study

was relatively better than the Newton study but there was no evidence that the results in the Newton study were unreliable. Several effects were identified in these studies and they could be attributed to methyl bromide exposure with the degree of adversity depending on the judgement of the authors/reviewers. The uncertainty in the data, however, could be attributed to two main factors: few dogs per group, and the use of concentrations close to the NOEL. In this section, the magnitude of the NOELs from these two studies was compared. A summary of the NOELs and the proposed critical NOEL is presented in Table 7.

In the Newton study, a NOEL was not experimentally determined because the lowest dose, 5 ppm, was the LOEL for the study. In the 1999 RCD, a default factor of 10-fold⁵ was used to estimate the NOEL of 0.5 ppm because there were no data to determine the appropriate factor for the extrapolation. With the results from the Schaefer study where no effects (or few effects at 10 ppm and 20 ppm), it was clear that the 5 ppm was near the actual NOEL for methyl bromide subchronic toxicity. Therefore, the extrapolation factor could be reduced from 10-fold to 3-fold resulting in an estimated NOEL (ENEL) of 1.7 ppm for the Newton study. Both DPR and U.S. EPA have used a 3-fold factor in the extrapolation of a LOEL to a NOEL when the effects at the LOEL were considered mild or at a relatively low response rate.

For the Schaefer study, several NOELs have been identified: <5 ppm, 5 ppm, 10 ppm, and 20 ppm depending on the authors/reviewers' scientific judgment on the significance of the findings. If the Schaefer study were considered negative with a NOEL of 20 ppm, as indicated by the study author and Chambers (2002), then the NOEL for the study would be at 20 ppm. This NOEL would be higher than the ENEL of 1.7 ppm from the Newton study. The 20 ppm would not be selected as the critical NOEL since in weight of evidence, negative studies generally have less weight than studies with positive findings which were consistent with known effects for the chemical and with similar study limitations (few number of dogs studied, for example). If the 5-ppm or 10-ppm dose were the NOEL for the Schaefer study, based on the absence of proprioceptive placing response (Patterson, 2002; Pinkerton, 2002), these NOELs would still be higher than the ENEL of 1.7 ppm from the Newton study. If the 5 ppm was the LOEL for the Schaefer study, then this LOEL would support the LOEL determined for the Newton study and provide a stronger support for establishing the critical NOEL based on a LOEL of 5 ppm.

In comparison to the effects noted in the dog studies, the next most sensitive endpoint was reduced brain weight observed only in rodents (Table 8). As stated in the Neurotoxicity Guidelines, reduction of absolute brain weight is an adverse effect in itself and should not be dismissed in the presence of reduced body weight because brain weight is generally unaffected by body weight changes (U.S. EPA, 1998). For methyl bromide, reduction in brain weight may or may not be associated with neurotoxicity. In the 2-week studies, severe neurotoxicity and death were observed at the same dose as that for brain weight reduction (160 ppm, the only dose

⁵ The extrapolation factor for LOEL to NOEL is based on data analysis of toxicity studies which showed that the ratio between LOAEL to NOAEL was 10-fold or less (Dourson and Stara, 1983; Dourson *et al.*, 1996). This is primarily due to the dose spacing used in toxicology studies. In any case, this range has been adopted for LOEL to NOEL extrapolation. The specific value used depends on the scientific judgment of the data regarding adequacy of the database, the dose-response relationship and severity of effects. When data are absent or inadequate to determine such a factor, the default is a 10-fold factor. A 10-fold factor can also be used when the effect at the LOEL is considered severe. A smaller factor, generally a 3-fold factor, is used when the effect is less severe and the NOEL is assumed to be close to LOEL.

tested) in both rats and mice (Table 4) (Eustis *et al.*, 1988). In other rat studies, brain weight reduction occurred at lower doses than those for neurobehavioral effects (NTP, 1992; Eustis, 1992; Norris *et al.*, 1993 a and b). There was an apparent strain difference for this endpoint. CD rats showed brain weight reduction at 30 ppm with mortality and clinical signs at 70 and 140 ppm (Norris *et al.*, 1993 a and b). For Fischer-344 rats, brain weight was reduced at 120 ppm with few neurobehavioral effects (NTP, 1992; Eustis, 1992). The lowest LOEL for brain weight reduction was 30 ppm (Norris *et al.* 1993 a and b) (Table 6). The 30-ppm was the lowest dose tested with the brain weight significantly reduced ($p < 0.01$) at 96% of control. Using a default extrapolation factor of 10-fold, the estimated NOEL was 3 ppm. This ENEL was 2-fold higher than the ENEL of 1.7 ppm from the Newton study. But in terms of human equivalent NOEL⁶, it was 1.1 ppm, 4-fold higher compared to the human equivalent NOEL of 0.3 ppm for the Newton study.

Table 7. No-Observed-Effect Levels for methyl bromide subchronic toxicity.

Studies	Species/ Duration	Effect	NOEL/ LOEL (ppm)	ENEL ^a (ppm)	Human Equivalent NOEL ^b	Reference concentration ^c
Subchronic Exposure (6-13 weeks)						
Newton, 1994a	Dog/ 6 weeks	Unrespon- siveness	<5 / 5	0.5 UF=10	0.1 ppm	1 ppb
				1.7 UF=3	0.3 ppm	3 ppb
Schaefer, 2002	Dog/ 6 weeks	Tremors, twitching, emesis	<5/5	1.7 UF=3	0.3 ppm	3 ppb
		Absence of Proprio- ceptive-placing response	5 / 10	NA	1 ppm	9 ppb
		No Effects	20/ >20	NA	4 ppm	36 ppb
Norris <i>et al.</i> , 1993 a and b	Rat/ 13 weeks	Brain weight reduction	<30/30	3 UF=10	1.1 ppm	11 ppb

a/ In the absence of a NOEL, the LOEL is divided by an uncertainty factor (UF) to estimate a NOEL.

b/ Human equivalent NOELs take into consideration of respiratory rate differences between experimental animals and humans (based on children rate of 0.46 m³/kg/day for all cases except for developmental toxicity where the adult respiration rate of 0.26 m³/kg/day was used) and amortized for 24 hours of exposure.

c/ Reference concentration is 1/100 of the human equivalent NOEL.

⁶ Human equivalent NOEL calculation takes into consideration of respiratory rate differences between experimental animals and humans (based on children rate of 0.46 m³/kg/day for all cases except for developmental toxicity where the adult respiration rate of 0.26 m³/kg/day was used) and amortized for 24 hours of exposure.

Table 8. Summary of methyl bromide effect on brain weight and neurotoxicity.^a

Species/ Duration	NOEL/LOEL (ppm)	NOEL/LOEL (human equivalent, ppm) ^b	Effect	Ref. ^a
Short-term and Subchronic Toxicity				
Rat/2 weeks	<160/160	<60/60	Brain wt 94% (M, p<0.05) death	1
Mouse/2 weeks	<160/160	<112/112	Brain wt 96% (M, p<0.05) 93% (F, p<0.001) of C; neurobehavioral effects and brain lesions	1
Rat/6 weeks	<160 /160	<60/60	Brain wt- 90% (F) of C (p<0.001) neurotoxicity, brain lesions, death	1
Rat/ 13 weeks	60/120	22/45	Brain wt 92/93% of C (p<0.01), few neurobehavioral effects	2,3
Rat/13 weeks	<30/30	<11/11	Brain wt 96% of C (p<0.01, F), neurobehavioral effects at 70 ppm and 140 ppm, brain lesions at 30 ppm (one animal) and 140 ppm	4
Mouse/13 weeks	80/120	56/84	Brain wt 92 (F) to 94% (M) of C (p<0.01), few neurobehavioral effects	2,3
Dog/ 6 week	103/>103 (<5/ 5)	20/>20 (<1/1)	No effect on brain weight (Decreased responsiveness)	5
Dog/6 weeks	20/>20	4/>4	No effects at HDT	6

^{a/} References: 1. Eustis *et al.*, 1988; 2. NTP, 1992; 3. Eustis, 1992; 4. Norris *et al.*, 1993 a and b; 5. Newton, 1994a; 6. Schaefer, 2002. wt=absolute organ weight.

^{b/} Human equivalent NOELs take into consideration of respiration rate differences between experimental animals and humans (based on children rate of 0.46 m³/kg/day and amortized for daily exposure.

IV. Conclusion

For the subchronic toxicity of methyl bromide, the proposed critical NOEL, as an ENEL, was 1.7 ppm (human equivalent NOEL of 0.3 ppm) based on the decreased responsiveness with a LOEL of 5 ppm from the Newton study (1994a). The finding of unresponsiveness in the dogs remained the most sensitive and appropriate endpoint for risk characterization. This same endpoint that was used in the Risk Characterization Documents (DPR, 1999; 2002a) but with a lower extrapolation factor (10-fold instead of 3-fold) to estimate the NOEL from a LOEL. Reevaluation of the database, including the Schaefer study, showed no evidence that the Newton study should not be considered in the overall weight of evidence to determine the critical NOEL. The data from the Schaefer study, as interpreted by some reviewers, actually confirmed that methyl bromide induced toxicity at low doses (near 5 ppm). Studies with rats also indicated a NOEL of less than 5 ppm for neurotoxicity because the lowest ENEL was 3 ppm (human equivalent of 1.1 ppm) for brain weight reduction. The revised ENEL for the Newton study resulted in a reference concentration (3 ppb) that is 3-fold higher than that (1 ppb) previously determined in the Risk Characterization Documents (DPR, 1999; 2002a).

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Appendix F

External Review by Dr. J. Last, University of California at Davis

Jay Schreider, Ph.D.
Primary State Toxicologist
Medical Toxicology Branch
Department of Pesticide Regulation

February 19, 2003

RE: Task Order #38-1

Dear Dr. Schreider,

As per task order #38-1, I was asked to provide a peer review of several documents related to the risk characterization performed by the Department of Pesticide Regulation for methyl bromide. The peer review was asked to focus on two documents, the 6 week inhalation study of methyl bromide in dogs performed by Schaefer (2002), and the 2002 addendum to the risk characterization document for methyl bromide prepared by the DPR. A list of specific questions to be addressed was included in the task order. All of the questions are specifically addressed in the narrative below with one exception. I did not try to address the issue of whether there is sufficient evidence of idiopathic necrotizing arteritis in one of the beagles exposed to 5ppm of methyl bromide to discount the neurological signs found in that animal as being related to exposure to methyl bromide. The material available for my review was not sufficient to allow a definitive answer to that question, nor do I have the requisite veterinary medical expertise to diagnose such an illness were it to have been present. This issue does not effect my conclusions presented at the end of my report. In my opinion the DPR-generated addendum is scientifically sound, and the Schaefer (2002) study is also scientifically sound and was appropriately conducted. The issues are those of interpretation rather than faulty science.

Methyl bromide is a fumigant used to control insects, nematodes, rodents, and other organisms in soil and commodities, as well as in building structures. Large quantities are used in California, especially as a soil fumigant, with an average of 5-10,000 tons per year (about 10-15% of total annual world use of this chemical) used for this purpose between 1991-1997. Grapes, almonds, flowers, and especially strawberries are major crops in California that depend on this fumigant. Since methyl bromide is a major ozone-depleting agent in the stratosphere, it is theoretically scheduled for phase out of its use in the United States by 2005 under the United Nations Montreal Protocol. However, the EPA issued a Federal Register Notice (5/10/02) announcing the availability of the Methyl Bromide Critical Use Exemption application. Critical Use Exemption would provide users of methyl bromide the opportunity to maintain uses, past the 2005 phase out, that do not have a technical and economically viable alternative.

Human exposure and health effect data are insufficient to use as the basis for standard setting. The most frequently cited human study was performed by Anger et al. (1986). Quoting from the U.S. EPA IRIS site, "In a cross-sectional occupational study, soil and structural fumigators underwent a neurological examination. The exposure group was blinded to the physician giving the examination. Most of the structural fumigators used both bromomethane (MB) and sulfuryl fluoride (SF). The formation of the study groups was based on the estimated time devoted to bromomethane and sulfuryl fluoride fumigation activities, and estimated length of time in the occupation. Four groups were formed: the MB group (n=32) consisted of structural fumigators using MB 80% or more of the time and soil fumigators using the mixture MB and chloropicrin; the SF group (n=24) consisted of structural fumigators who used SF 80% or more of the time. Group COMB (n=18) consisted of workers using both MB and SF 40-60%

of the time, and a reference group (Group R, n=29) consisted of those workers who were not directly exposed to fumigants, but worked in the fumigation industry. The workers in the exposed groups had been in the profession for 1 or more years and had fumigated a house or field within the last 50 days. More symptoms were reported in the exposed groups than in the reference population: 78-83% and 41%, respectively, showed symptoms. The difference was significant for the MB and COMB groups when compared to Group R. The MB group did not perform as well as referents on several behavioral tests, including tests of cognitive function, reflexes, sensory and visual effects. Although this study suggests mild neurological effects of exposure to methyl bromide, it is difficult to draw any conclusions between exposure and effect because of the confounding factors. The exposed and reference groups were not well matched for age; use of prescription medication, alcohol, or illegal drugs within 2 days of the testing; education; or ethnic group. In addition, participation in the study was voluntary and no information is provided on the use of personal protective equipment in these groups.” For our purposes, what is noteworthy from this study is that small numbers of workers exposed to concentrations of methyl bromide estimated to be at or near 5ppm (range 0-8.6ppm, mean range, 2.3-4.5ppm) had signs and symptoms of neurotoxicity. While these data are far from definitive, and not themselves suitable for standard setting, they do support the necessity for a cautious interpretation of any animal data that suggest a standard significantly higher than 5ppm.

DPR requested a review of the basis for selection of the critical value for a NOEL for methyl bromide toxicity by sub-chronic inhalation. Several alternative studies and interpretations could have been chosen, and conflicting opinions exist with regards to the best choice. A NRC committee has made recommendations on this issue, and both DPR and an industry alliance have also interpreted the data. This report will first indicate the different data sets and the range of choices that are available, then add its own interpretation to this discussion.

Both DPR (2002) and the NRC (2000) have published risk characterization documents. The NRC study was based upon their review of an earlier draft risk characterization document prepared by DPR (1999). The 1999 DPR analysis (and, therefore, the NRC analysis) relied upon data from two dogs (at 5ppm of methyl bromide) from a study performed in 1994 (Newton, 1994). Based upon the NRC study recommendations, an additional (new) 6-week sub-chronic study in dogs was performed by a research laboratory under contract to the registrant, and new data became available to assist in the determination of a NOEL value by sub-chronic inhalation (Schaefer, 2002).

A key issue in the NRC analysis was how best to interpret the findings of an animal neurologist of less responsiveness (listlessness and quiescence) in two female dogs from the 5 ppm group, the lowest dose tested. If this was indeed a LOAEL, then a default assumption of a factor of 10 to extrapolate from LOAEL to NOEL would be a standard approach for risk assessment, and the NOEL would be assumed to be 0.5ppm. The newer study (Schaefer, 2002) was designed to attempt to more precisely determine whether there was a NOEL at or near 5 ppm of methyl bromide; groups of dogs were exposed for 6 weeks to 0, 5, 10, or 20ppm. An attempt was made to make the neurological testing more objective than in the previous study by use of a Functional Observation Battery of tests. Controversy arose as to the appropriate value for the NOEL from this study. The study authors established a NOEL of 20ppm by ascribing tremors and twitching observed in one of the dogs exposed to 5ppm as due to idiopathic febrile

necrotizing arteritis and not related to the treatment with methyl bromide. There was also a difference in interpretation of the significance of three dogs, one in the 10ppm group (#8732) and two in the 20ppm group (#8723 and #8751), not exhibiting the proprioceptive-placing response (a component of the FOB) in this study. The reviewer from DPR established a LOAEL of 5ppm in this experiment based upon the tremors and twitching in the same dog at 5ppm, and upon episodes of emesis in 3 of the 4 dogs tested at 5ppm. The small number of dogs studied clearly allows for reasonable people to disagree on the correct interpretation of this study based upon the findings in a single animal. The registrant requested an independent analysis of this study by Professor Janet Chambers (Mississippi State University), a member of the original NRC (2000) committee. She agreed with the registrant that 20ppm was the appropriate value for the NOEL based upon the Schaefer (2002) data. Based upon a subsequent DPR consensus (not unanimous) of their senior staff, and interpretation of the data by another consultant (Dr. Kent Pinkerton, University of California, Davis), the 5ppm value was suggested as a NOEL in this study. The DPR staff minority opinion was that 5ppm was a LOAEL. Clearly, with a possible factor of 10 default for LOAEL to NOEL extrapolation to be considered, this is a very important distinction for performance of a risk assessment.

What complementary data are there? Methyl bromide was tested for toxicity in rats and mice by the National Toxicology Program (1992). The acute study, at 160ppm, was at too high a dose to be relevant here. The sub-chronic study in rats for 13 weeks tested exposure groups at 0, 30, 60, or 120ppm. A NOEL for neurotoxicity was observed at 60ppm. Mice were tested for 13 weeks at 0, 10, 20, 40, 80, or 120ppm. A NOEL was reported at 80ppm for neurobehavioral effects. If we use the conventional default factor of 10 for interspecies extrapolation (assuming that dogs might be more sensitive to methyl bromide than rats and mice), then a predicted NOEL in dogs of 6-8ppm might be considered reasonable. It should be emphasized that these values are of no real significance; they merely suggest that a NOEL in dogs for neurotoxicity in the range of 5-10ppm of methyl bromide is a reasonable observation if we do nothing more than extrapolate from the reported findings in rats and mice. Similarly, if we take 10-20ppm as a LOAEL in dogs, which seems conservative but reasonable in light of the Schaefer (2002) study, and a default factor of 3 for the LOAEL to NOEL extrapolation, we would get a calculated NOEL value of 3-7ppm. One can suggest, based upon all of these guesses, extrapolations, and estimates, that there seems to be a weight of evidence argument for a NOEL of about 5ppm for methyl bromide. This value is consistent with the majority consensus of senior DPR staff and Dr. Pinkerton's interpretation of the results of the dog studies.

Before we can accept this conclusion, we are left with the issue of the proper interpretation of the apparent neurotoxic observations reported in dogs exposed to 5ppm of methyl bromide in the Schaefer (2002) study—emesis, twitching and tremors. While these findings can be interpreted as manifestations of neurotoxicity, there is no indication of similar responses in the dogs exposed to 10 and 20ppm, suggesting a lack of dose-response for these observations. The study director dismissed these findings as incidental and not treatment related (Schaefer, 2002). It should, however, be noted that there were also isolated observations of changes in proprioceptive-placing response, a component of the FOB testing, in one male dog at 10ppm and one dog of each sex at 20ppm. This seems a classic dilemma for the risk assessor: whether to err in the direction of being too conservative or in the direction of being too permissive when the only data available are not definitive. Generally, this is a common problem

when small numbers of animals are investigated at exposure concentrations at or near the LOAEL or NOEL value. It is clear that under these circumstances the issues are not strictly scientific, but are very much judgmental, and reasonable people can differ in their interpretation of the same findings.

From the point of view of the Alliance of the Methyl Bromide Industry, we are also left with the issue of why 20ppm is not an appropriate value for the NOEL, given the interpretations of the study director (Schaefer, 2002) and of Professor Chambers. To quote from a letter from William Thomas, a lawyer for the Alliance, commenting on the Newton study (1994), “A subjective observation of the 5ppm dogs after 6 week exposure period noted a decrease in responsiveness...in two female dogs. This observation was equivocal at best, and, in any event, the finding was not dramatic.” This reviewer does not believe that toxicity testing requires that findings be dramatic, merely that there be an observed response that might be adverse. Thus, the real issue here ought to be whether this observation was indicative of an adverse response. I will also quote from Dr. Chamber’s letter commenting on the Schaefer study (2002). “The only notable and consistent abnormalities were related to a single male dog in the 5ppm treatment group, and these abnormalities were consistent with Beagle pain syndrome; this animal did not concurrently show neurological dysfunction consistent with neurotoxicity since his FOB observations were normal. Therefore, the study’s conclusions that the abnormalities displayed by this animal were not attributable to exposure to methyl bromide are reasonable.” We are again in the situation of the preceding paragraph: whether it is better to err in the direction of being too conservative or in the direction of being too permissive when the only data available are not definitive. Once again the issues are not strictly scientific, but are very much judgmental, and reasonable people can differ in their interpretation of the same findings.

This reviewer believes that the equivocal nature of the findings in dogs between 5-20ppm of methyl bromide is exactly what would be expected when relatively small numbers of animals are exposed to a toxicant at concentrations at or near the NOEL value. The fact that the findings at 5ppm are subjective rather than objective is a commentary on the relative sensitivity of the assays, not a definitive rebuttal of the subjective observations. The fact that only one dog exposed to 10ppm, and two dogs exposed to 20ppm, of methyl bromide showed changes in only a single component of the FOB is also consistent with exposure to concentrations very close to the NOEL. The apparent positive results of the Anger et al. (1986) study of humans exposed to methyl bromide at concentrations near 5ppm are consistent with this conclusion, as are the isolated changes in FOB testing (proprioceptive-placing response) at 10 and 20ppm. In my opinion there is enough evidence, albeit equivocal, for neurotoxic effects in the study animals at or near 5ppm to suggest that it is best to err on the side of caution in this analysis. Thus, my recommendation is to use the value of 5ppm as a NOEL for all of the reasons cited above.

Sincerely,

Jerold Last, Ph.D.
Professor, Pulmonary and Critical Care Medicine