

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY  
DEPARTMENT OF PESTICIDE REGULATION  
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA

METHYL SALICYLATE

Chemical Code # 746, Tolerance # 50578  
SB 950 # 472

6/9/00

I. DATA GAP STATUS

Chronic toxicity, rat:	Inadequate study; possible adverse effect indicated <sup>1</sup>
Chronic toxicity, dog:	Inadequate study; no adverse effect indicated <sup>1</sup>
Oncogenicity, rat:	No study on file <sup>1</sup>
Oncogenicity, mouse:	No study on file <sup>1</sup>
Reproduction, rat:	Inadequate study; possible adverse effect indicated <sup>1</sup>
Teratology, rat:	Inadequate study; possible adverse effect indicated <sup>1</sup>
Teratology, rabbit:	No study on file <sup>1</sup>
Teratology, hamster:	Inadequate study; possible adverse effect indicated <sup>1</sup>
Gene mutation:	Inadequate study; possible adverse effect indicated <sup>1</sup>
Chromosome effects:	No study on file <sup>1</sup>
DNA damage:	No study on file <sup>1</sup>
Neurotoxicity:	No study on file <sup>1</sup>

Toxicology one-liners are attached.

All record numbers through 158835 were examined.

\*\* indicates an acceptable study.

Bold face indicates a possible adverse effect.

## indicates a study on file but not yet reviewed.

File name: T169887

<sup>1</sup>New active ingredient, Methyl Salicylate, submitted as a biochemical for terrestrial food use. These studies are not required at this time.

## II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

These pages contain summaries only. Individual worksheets may contain additional effects.

### CHRONIC TOXICITY, RAT

50578-003; 158833; "Chronic Oral Toxicity of Oil Sweet Birch (Methyl Salicylate)"; (E.W. Packman et. al.; LaWall and Harrisson Research Laboratories, Philadelphia, PA; Pharmacologist 3, pp. 62 (1961)); Albino rats (strain not identified) received 700 or 2100 ppm of methyl salicylate in the diet for 2 years. There were no treatment-related effects noted upon growth, survival, or food consumption. No effects were evident upon blood or urine parameters. No treatment-related lesions were noted in the pathological evaluation. No adverse effects indicated. **Summary report.** (Moore, 1/15/98)

50578-002; 158791; Published Toxicology Data (Acute Oral Toxicity: Rat; Acute Dermal Toxicity: Rabbit; and Primary Dermal Irritation:Rabbit) for Methyl Salicylate, Fragrance Raw Materials Monographs® (D.L.J. Opdyke; *Fd. Cosmet. Toxicol.* 16, pp. 821-825, (1978); Toxicity data for methyl salicylate were reported in the monograph. Acute oral LD50 values of 1110 (mouse), 887 and 1250 (rat), 1060 and 700 (guinea pig), 1300 and 2800 (rabbit), and 2100 (dog) mg/kg were reported. An acute dermal LD50 value (rabbit) exceeded 5000 mg/kg. When the test material was applied to the rabbit skin for 24 hours under an occlusive wrap, it was moderately irritating. The dose of material applied to the skin was not reported. Treatment-related signs in dogs dosed orally with between 0.6 and 4.7 g/kg of methyl salicylate were nausea, vomiting, intense hyperpnea, excitation of the nervous system and diarrhea. Dogs receiving orally 0.6 to 5 g/kg of the test material demonstrated increased respiratory amplitude without a change in arterial pressure. In another study, dogs treated orally with 700 mg/kg exhibited a decrease in blood pressure and cardiac output with an increase in heart rate within 5 hours of test material administration. These effects were attributed to hyperkalemic cardiomyopathy and inhibition of high energy phosphate production.

In a subacute study, rats which received a 1% dietary supplement of the test material for 13 days, demonstrated pronounced alterations of microbodies in their hepatocytes. In a chronic dietary study, rats received 0.1, 0.5, 1.0 and 2.0% supplements of the test material for up to 2 years. All of the rats in the 2% group had died by week 50. An increase in cancellous bone was noted in this group as well as to a lesser extent in the 1.0% group. A ten week feeding study in which rats were treated with 20000 or 11250 ppm of the test material, also demonstrated an increase in cancellous bone in the tibia and femur. At doses of 9000 ppm or less, this effect was not noted. Oral administration of methyl salicylate at doses of 50 to 1200 mg/kg/day, 6 days/week for 59 days to dogs resulted in weight loss and death in all dogs receiving doses of 500 mg/kg/day and greater. Dogs in the 800 and 1200 mg/kg/day treatment groups demonstrated fatty metamorphosis of the liver. In a two year study, dogs treated orally with 150 or 350 mg/kg/day exhibited enlarged livers with an increase in the size of hepatic cells. Rabbits treated dermally with 4 ml/kg/day of the test material for 90 days demonstrated signs of kidney damage sufficiently severe as to result in death. At lower doses, the animals were predisposed to spontaneous nephritis and mild hepatitis.

Female rats treated ip with 0.1 ml of the test material on gestation days 10 and 11 had fewer and smaller offspring, and more resorptions and malformed young than the controls did. Treatment with the test material retarded renal development in the fetuses. In another study, sc administration of the test material to female rats on gestation days 9 or 10 resulted in cardiovascular abnormalities in the fetuses. Likewise, in a third study, in which female rats were treated sc on gestation days 7, 9 or 11, anomalies in the palate and tail of the offspring were reported along with an increase in fetal resorptions and a decrease in survival rate and body weights of the fetuses. In a 3 generation rat reproduction study, treatment with 3000 or 5000 ppm of the test material in the diet resulted in significant decreases in mean litter size and the mean number of live-born progeny, survivors to day 4 and survivors to weaning.

Metabolically, methyl salicylate is converted to salicylic acid in the body. Rats and dogs appear to hydrolyze methyl salicylate more readily than do humans. The major site of hydrolysis is the liver. Certain steroids known to induce hepatic microsomal drug metabolizing enzymes prevented severe methyl salicylate poisoning. However, other microsomal inducing agents were without effect or exacerbated the toxicity. In humans, 4 to 8 ml of methyl salicylate is considered to be a potentially lethal dose for children. Signs of such poisonings include nausea, vomiting, acidosis, pulmonary edema, pneumonia and convulsions. Death from respiratory failure is preceded by a period of unconsciousness. (Moore, 1/12/98)

**50578-003; 158826;** "Chronic and Subacute Toxicology and Pathology of Methyl Salicylate in Dogs, Rats, and Rabbits"; (W.K. Webb and W.H. Hansen; Division of Pharmacology, Food and Drug Administration, Dept. of HEW, Washington, D.C.; Toxicol. Appl. Pharmacol. 5, pp. 576 - 587 (1963)); Rats, dogs and rabbits were used as test animals to evaluate the toxicity of methyl salicylate (99% pure). Osborne-Mendel rats were fed diets containing 0, 0.1, or 1.0% for 17 weeks. The mean weight gain was significantly lower ( $p < 0.001$ ) in the high dose group. In a succeeding chronic feeding study, rats were fed diets containing 0, 0.1, 0.5, 1.0, or 2.0% of the test material for 2 years. None of the animals in the 2.0% group survived beyond 49 weeks. The mean body weights were significantly less (p value not reported) for the 1.0% group over the course of the study. A greater number of pituitary lesions were noted grossly in the 0.5% group than in the control animals (10 to 4). There was a dose-related effect on the incidence and severity of cancellous bone noted in the treatment groups (0.5 and 1.0% groups). In the affected bones, there were fewer osteoclasts. In a supplemental study, rats fed a 2% diet of the test material for up to 71 days were evaluated for the effects noted in the bone. Each time one of the treatment animals died, a concurrent control was also euthanized. An increased area of dense bone in the growth areas of all bones was noted for the treated animal surviving to 71 days. The growth plate grew more slowly than normal, resorption of cartilage by the hypertrophic cartilage cells decreased, the primary trabeculae became progressively thicker, and the primary trabeculae were not resorbed as expected. In addition, gastric hemorrhages were noted in the treated animals.

In a subacute protocol, one dog/sex was treated orally with 0, 50, 100, 250, 500, 800, or 1200 mg/kg/day of methyl salicylate in capsules, 6 days/week for 59 days. Dogs receiving the two highest doses vomited within hours of receiving each of their doses. The dogs in the 500 mg/kg/day and higher groups died or were euthanized within a month. Fatty metamorphosis of the liver was noted in the two highest dose groups. In a chronic oral toxicity study, two beagles/sex/group were treated orally with 0, 50, 150 or 350 mg/kg/day of the test material in capsules, 6 days/week for 2 years. Animals in the two higher dose groups demonstrated a dose-related effect upon mean body weight. The dogs in both these groups suffered from enlarged livers. Hepatic cells were enlarged in these dogs.

A subchronic dermal toxicity study was performed by treating 3 rabbits/group (number/sex not reported) with 0, 0.5, 1.0, 2.0 or 4.0 ml/kg/day, 6.5 hours/day, 5 days/week for 96 days. All of the rabbits in the 4.0 ml/kg/day died by day 28. Lesions were noted in the kidneys of these animals. The incidence of spontaneous nephritis and mild hepatitis was greater in the treated animals than the incidence noted in control animals. **Possible adverse effect:** altered bone growth in the rat. **Summary Study.** (Moore, 1/14/98)

#### CHRONIC TOXICITY, DOG

See Record No. 158791 above.

See Record No. 158826 above.

#### ONCOGENICITY, RAT

No study submitted.

#### ONCOGENICITY, MOUSE

No study submitted.

## REPRODUCTION, RAT

**50578-003; 158831**; “Results and Evaluations of 48 Continuous Breeding Reproduction Studies Conducted in Mice”; ( R.E. Morrissey et. al.; Developmental and Reproductive Toxicology Group, NIEHS, NTP Program, Research Triangle Park, NC, Analytical Sciences Inc., Durham, NC, Environmental Health Research and Testing, Inc., Lexington, KY; Fund. Appl. Toxicol. 13, pp. 747-777 (1989)); The reproductive toxicity of methyl salicylate was evaluated using the NTP Reproductive Assessment by Continuous Breeding study protocol. In this protocol, dosing with the test material was initiated 1 week prior to individual males and females being cohabited. This cohabitation continued for the next 14 week dosing period. Litters were removed as the offspring were born. After 14 weeks, the last litter remained with the mother until weaning. At this time, the parents were crossmated with control animals and evaluated for effects upon reproduction. Treatment was continued for the second generation offspring and they were mated with members of their respective treatment groups. Two studies were performed in which CD-1 mice in the first study were treated orally with 0, 0.10, 0.25, or 0.50 g/kg/day and in the second study were treated with 0, 0.025, 0.05, or 0.10 g/kg/day. At 0.50 g/kg/day, the mean number of litters/pair, mean number of live pups/pair, proportion of pups born alive and the mean live pup weight/litter were all significantly decreased over the control values ( $p<0.05$ ). The adjusted mean live pup weight/litter was decreased for the 0.25 and 0.50 g/kg/day treatment groups ( $p<0.05$ ). No effects upon reproduction were noted in the second generation group treated with 0.10 g/kg/day. Likewise, no effects were noted for the parents in the first study cross mated with control animals. **Possible adverse effect:** significant decrease in particular reproductive parameters. **Summary Report.** (Moore, 1/15/98)

**003; 158834**; “Methyl Salicylate: Fertility Assessment in CD-1 Mice when Administered by Gavage”; (D.K. Gulati and H. Choudhury; Environmental Health Research and Testing, Cincinnati, OH; Project No. N01-ES-2-5013; 1/4/85); The effect of methyl salicylate upon the fertility of CD-1 mice was assessed using the Fertility Assessment by Continuous Breeding study protocol. In this protocol, mice were dosed orally for two weeks with 0, 0.05, 0.1, 0.25, 0.5 or 1.0 g/kg/day with the test material in order to establish a maximum tolerated dose (Task 1). In the continuous breeding phase (Task 2), mice were dosed orally by gavage with 0, 0.1, 0.25 or 0.50 g/kg/day for 18 weeks. There were 40 animals/sex in the control group and 20 animals/sex/group in the treatment groups. After the first week, individual males and females for each group were housed together for the next 14 weeks, followed by an additional three weeks of treatment. At the conclusion of this period, animals in the 0.50 g/kg/day group were cross mated with control animals in order to determine which sex may have been affected by the treatment (Task 3). During the cohabitation period of 7 days, no treatment was performed. Otherwise, the animals were dosed with the test material for 7-8 weeks between trial #1 and #2 and after the second mating period for 7 weeks. Two of the animals in the 1.0 g/kg/day group apparently died as a result of the treatment (Task 1). In Task 2, the mean number of litters/pair (0.5 g/kg/day:  $p<0.05$ ), mean number of live pups/litter (0.5 g/kg/day:  $p<0.01$ ), percentage of pups born alive (0.5 g/kg/day:  $p<0.05$ ), mean live pup weight (0.25 g/kg/day, females,  $p<0.05$ ; 0.5 g/kg/day, combined,  $p<0.05$ ), and adjusted mean live pup weight (0.25 g/kg/day, female and combined,  $p<0.05$ , 0.5 g/kg/day, male, female, combined,  $p<0.01$ ) were all reduced. Despite two trials being performed in Task 3, no judgement as to the adversely affected sex could be determined due to the poor fertility demonstrated by the control animals. **Possible Adverse effect:** reduction in number of litters per pair, mean number of live pups/litter, and mean pup weight at 0.5 g/kg/day. **Reproductive NOEL:** 0.1 g/kg/day (based upon reduction in adjusted mean pup weight for the 0.25 g/kg/day group). **Study supplemental.** (Moore, 1/20/98)

**50578-003; 158835**; “Effect of Methyl Salicylate on Rat Reproduction”; (T.F.X. Collins, et. al.; Bureau of Foods and Pesticides, Food and Drug Administration, U.S. Dept. of Health, Education and Welfare, Washington, D.C.; Toxicol. Appl. Pharmacol. 18, pp. 755-765 (1971)); Methyl salicylate was fed in the diet to Osborne-Mendel rats (Fo), 20 animals/sex/group, at doses of 0, 500, 1500, 3000 or

5000 ppm for 100 days, mated twice, with both litters (F1a and F1b) being weaned. Twenty animals/sex/group from the second litter (F1b) were then mated twice as well with both litters (F2a and F2b) being weaned. A third round of mating was performed in which 20 F2b weanlings were mated twice and the F3a and F3b litters weaned. A supplemental study was performed in which 20 F2b weanlings/sex/group were treated with 1500 ppm calcium carbonate in addition to the methyl salicylate in the diet and mated twice and their offspring being weaned. No treatment-related effect was noted in the parental animals. Although no statistically significant decrease in fertility was noted, the fertility index was lower in the 5000 ppm treatment group for the 2nd and 3rd generations. Significant decreases in the mean litter size were evident for the 2nd generation (3000 ppm, F3b litter,  $p < 0.05$ , and 5000 ppm, F3a and F3b,  $p < 0.01$ ). However, this effect was not noted for the 1st or 3rd generation matings. The mean number of liveborn pups per mated female was significantly reduced for the 2nd generation (F3a and F3b, 3000 ppm,  $p < 0.05$ , 5000 ppm,  $p < 0.01$ ). This effect was not evident for the 1st or 3rd generation matings. Likewise, the mean number of offspring which survived to day 4 per mated female was significantly reduced for the same groups in the second generation matings and not in the other two matings. The calcium supplement did not particularly alter the treatment-related effects noted for methyl salicylate. **Adverse effect:** reduction in litter size and number of live born pups/mated female. **Parental NOEL:** 5000 ppm; **Reproductive NOEL:** 1500 ppm (based upon reproductive effects in the 3000 ppm group); **Summary Report.** (Moore, 1/20/98)

#### TERATOLOGY, RAT

**50578-003; 158829;** "Evaluation of the Dermal Absorption and Teratogenic Potential of Methyl Salicylate in a Petroleum-based Grease"; (R. Infurna et. al.; Exxon Biomedical Sciences, Inc., East Millstone, NJ; Teratology 41, pp. 566 (1990)); Pregnant rats were exposed dermally to 0, 1, 3 or 6 g/kg/day to a petroleum-based grease containing 3% methyl salicylate (PBG/MS) on gestational days 6 - 15. A positive control was used in which animals were treated dermally with 2 g/kg/day with methyl salicylate on gestational days 6-9 and with 1 g/kg/day on gestational days 10-15. The dose was reduced due to excessive maternal toxicity (25% mortality and severe dermal irritation). In the positive control, there was total resorption of all of the embryos. The urinary concentration of salicylic acid for the positive control animals was reported as being quite high. Salicylic acid was recovered from the urine of the animals treated with the test compound but at much lower quantities. No signs of maternal or fetal toxicity were noted in the animals. **Maternal NOEL=Developmental NOEL for PBG/MS** >6 g/kg/day (based on no effects at HDT); **Possible adverse effect:** total resorption of embryos noted in the positive control (undiluted methyl salicylate, 2 g/kg/day). **Summary Report.** (Moore, 1/15/98)

#### TERATOLOGY, RABBIT

No Study submitted.

#### TERATOLOGY, HAMSTER

**50578-003; 158828;** "Comparative Teratogenic Effects of Methyl Salicylate Applied Orally or Topically to Hamsters"; (D.O. Overman and J.A. White; Department of Anatomy, West Virginia University, Morgantown, WV; Teratology 28, pp. 421-426 (1983)); Pregnant hamsters were dosed orally or exposed dermally to methyl salicylate on the 7th day, 9th hour of gestation. The animals were dosed orally by gavage with 1.75 g/kg of the test material. Dermally, the animals were exposed for 2 hours to 3.50 or 5.25 g/kg of methyl salicylate. Embryos were recovered at 9 days of gestation and evaluated for defects in neural tube closure. For the orally dosed group, 72% of the embryos were noted as suffering neural tube defects as compared to 11% in the control group. For the dermally exposed animals, 6 and 53% of the embryos suffered the defects in the 3.50 and 5.25 g/kg groups, respectively. The control group embryos were free of any neural tube defects. The oral dose resulted in a peak plasma level of

salicylate of 120 mg/100 ml at 2 hours post-dose. The peak plasma levels of salicylate after dermal application of 3.50 g/kg of the test material was 50 mg/100 ml at 5 to 6 hours post-application. **Possible adverse effect:** increased incidence of neural tube defects. **Summary report.** (Moore, 1/15/98)

#### GENE MUTATION

50578-003; 158802; "Published Toxicology Data (Genotoxicity) for Methyl Salicylate: *Salmonella* Mutagenicity Tests: II. Results from the Testing of 270 Chemicals"; (K. Mortelmans et. al.; *Environmental Mutagenesis*, Volume 8, Supplement 7: 1-119 (1986); Methyl salicylate was among 270 chemicals assayed for their mutagenic potential by means of the Ames *Salmonella* mutagenicity test. *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 were preincubated for 20 minutes with shaking followed by plating and incubation for 48 hours at 37° C with 1.0 to 333.3 µg/plate of methyl salicylate under conditions of +/- activation with triplicate plates per concentration. Activation was accomplished using an Arochlor 1254 induced S-9 fraction derived from either rat or hamster liver. There was no treatment-related increase in the number of revertant colonies/plate. Data presented in summary tables. **Summary Report.** (Moore, 1/13/98)

**50578-003; 158803;** "Mutagenicity of Analgesics, Their Derivatives, and Anti-Inflammatory Drugs with S-9 Mix of Several Animal Species"; (N. Kuboyama and A. Fujii; Department of Pharmacology, Nihon University School of Dentistry at Matsudo, Matsudo, Chiba 271, Japan; J. Nihon Univ. Sch. Dent. Vol. 34, pp. 183 to 195 (1992)); The mutagenic potential of methyl salicylate was evaluated in the Rec-assay with *Bacillus subtilis* strains H17 (Rec<sup>+</sup>) and M45 (Rec<sup>-</sup>) and in the Ames assay with *Salmonella typhimurium* strains TA98 and TA100. In the former assay, 0.25 to 5 mg/disc of the test material was incubated for 24 hours at 4° C and then for 24 hours at 37° C on plates on which a streak of inoculum from each strain of bacteria had been preplated. Difference in the extent of inhibition of growth of the Rec<sup>+</sup> strain as compared to that of the Rec<sup>-</sup> strain was the criteria for determining whether the test material was genotoxic. In the Ames assay, 0.1 mg/plate of the test material was incubated for 48 hours at 37° C in the presence or absence of activation. Activation was accomplished using a polychlorobiphenyl-induced S-9 fraction derived from either the rat, mice, guinea pig or hamster liver. A greater than two fold increase in the number of revertant colonies/plate above that of the control was considered to be a positive assay for mutagenicity. In samples activated with the hamster hepatic S-9 fraction, both the TA98 and TA100 strains demonstrated a greater than two fold increase in revertant colonies. No treatment-related effect was noted in the Rec-assay or for the non-activated cultures and those activated with either rat, mouse or guinea pig S-9 liver fractions in the Ames assay. **Indicated Adverse Effect. Summary Report.** (Moore, 1/14/98)

#### CHROMOSOME EFFECTS

No study submitted.

#### DNA DAMAGE

No study submitted.

#### NEUROTOXICITY

No study submitted.

#### METABOLISM STUDIES

See Record No. 158791 above.

50578-002; 158792; "On the Metabolism and Toxicity of Methyl Salicylate"; (C. Davison et. al.; Dept. of Pharmacology, The George Washington University School of Medicine, Washington, D.C.; 10/31/60;

J. Pharmacol. and Exper. Therapeut. 132, pp. 207-211 (1961)); The oral LD50 values for the mouse are 1110 mg/kg for methyl salicylate and 1070 mg/kg of sodium salicylate. In the rat, when animals were treated with the same equivalents of salicylic acid orally, plasma and brain levels of salicylic acid were equivalent at 20 and 60 minutes after dosing whether the animals were treated with methyl salicylate, sodium salicylate or acetyl-salicylic acid. In the dog, 95% of the methyl salicylate was hydrolyzed within 1 hour after dosing. For humans, methyl salicylate is less readily hydrolyzed with 21% of the total salicylate recovered 90 minutes after dosing being unmetabolized. In the rat, the liver was determined to be the primary site of hydrolysis. Diisopropylfluorophosphate completely inhibited the hydrolytic activity. The authors posed the question as to whether the reduced level of hydrolysis in humans may present a greater toxic potential with greater sequestration of the lipid-soluble test material in various lipid rich tissues such as the brain. **Study supplemental.** (Moore, 1/12/98)