HEALTH CONSULTATION

DACTHAL® GROUNDWATER CONTAMINATION

ADDITIONAL TOXICOLOGICAL DATA

COLOMA TOWNSHIP, BERRIEN COUNTY, MICHIGAN

Prepared by

Michigan Department of Community Health
Under a Cooperative Agreement with the
Agency for Toxic Substances and Disease Registry

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Summary

In a previous health consultation, the Michigan Department of Community Health (MDCH) determined that the groundwater contamination in Coloma Township, Michigan, by the herbicide Dacthal® and its metabolites constituted a public health hazard. Refined analyses of groundwater samples from the township have since confirmed that the samples contained not Dacthal® but its di-acid metabolite 2,3,5,6-tetrachloroterephthalic acid (TPA). The current manufacturer of Dacthal® subsequently provided toxicological information for TPA to MDCH.

Based on animal and *in vitro* data, TPA-contaminated groundwater poses an indeterminate public health hazard. There are no screening-level values for TPA, and the database for the compound may not be adequate to derive such values. Longer-term studies may be necessary to assess human health implications of exposure to TPA in drinking water.

MDCH recommends that the U.S. Environmental Protection Agency consider establishing analytical methods specific for Dacthal® metabolites. Regulatory agencies should analyze environmental samples for both the parent compound and its metabolites to determine which contaminant is present. If TPA is the contaminant of concern, MDCH recommends that the manufacturer conduct additional studies on this compound and provide the results to state and federal regulatory and health agencies. The agencies should consider establishing a screening level for TPA.

Purpose and Health Issues

The purpose of this document is to provide follow-up to the health consultation, "Dacthal® Groundwater Contamination, Coloma Township, Berrien County, Michigan" (ATSDR 2002). Under a cooperative agreement with the federal Agency for Toxic Substances and Disease Registry (ATSDR), MDCH prepares health consultations for sites of environmental contamination.

Since the release of the previous health consultation for this site, MDCH has received confirmatory analytical results and additional toxicological information on the di-acid metabolite of Dacthal®, TPA. This information was not available when the earlier health consultation was completed. MDCH has evaluated the new information and provides comment on it here.

Background

The herbicide Dacthal® (dimethyl tetrachloroterephthalate) was first detected in groundwater samples from Coloma Township, Berrien County, Michigan (Figure 1) in 2001 at Washington Elementary School. The school had been randomly chosen along with others in the state for a U.S. Environmental Protection Agency (EPA) non-community water supply study. The Michigan Department of Environmental Quality

(MDEQ) found widespread groundwater contamination in the township (ATSDR 2002). The Michigan Department of Agriculture (MDA) investigated the possible source of contamination but could not find a responsible party (R. Pigg, MDA Groundwater Monitoring Program, personal communication, 2003).

Based on the presence and persistence of Dacthal® metabolites in the environment, MDA determined that Dacthal® "is likely to cause an unreasonable adverse effect on the environment, including contamination of groundwater" at sites throughout the state of Michigan. As a result, MDA planned to cancel the state registration of pesticides containing Dacthal® (MDA 2003). Upon notification that the cancellation of the herbicide would occur, Amvac Chemical Corporation, the current manufacturer of Dacthal®, met with MDA, MDEQ, and MDCH. The company argued that the groundwater contaminant was the di-acid, TPA, that it was less toxic than the parent compound, and provided toxicological data on the metabolite. Since that meeting, MDEQ and MDA have confirmed that the compound present in the groundwater samples from Coloma, and from another site of contamination in the state, is TPA (C. Rubitschun, MDEQ Water Division, personal communication, 2003; L. Schmelzer, MDA, personal communication, 2003).

Discussion

Toxicological Evaluation

A detailed evaluation of the animal and *in vitro* studies on TPA is provided in Appendix A. According to the company, there are no other studies on the mono- or di-acid (A. Manley, Amvac Chemical Corporation, personal communication, 2003). Deficiencies in the studies conducted in the 1960s preclude their value in determining a screening value for TPA. Compared to the later animal studies, the 1977 study by the International Research and Development Corporation has weak statistical value. Only a few deficiencies were noted in the 1985 rat studies.

After a no-observed or lowest-observed adverse effect level (NOAEL or LOAEL) is determined from a key research study, numbers called "uncertainty factors" are applied to that value in order to achieve an acceptable level of protection. These factors attempt to account for converting the dose from a LOAEL to a NOAEL (if none of the doses in the key study resulted in no adverse effects), extrapolating animal results to possible human health effects, accounting for a study that was less-than-lifetime (subchronic) to long-term (chronic), and protecting sensitive subgroups within a population, such as children or those whose immune system is impaired. An uncertainty factor also may be applied to address the adequacy of the database for a compound. According to Dourson (1994), a "complete" database includes two adequate mammalian (different species) chronic studies by an appropriate exposure route, two adequate mammalian (different species) developmental studies by an appropriate exposure route, and one adequate mammalian multigeneration reproduction study by an appropriate exposure route. Neither the oral-intubation nor the teratology study of TPA was a chronic study. Only rats were used in those experiments. Therefore, the TPA database is not complete, and a screening level value would likely have much uncertainty in its derivation. Further research may be

needed to improve the database and decrease the uncertainty if regulatory or health agencies were to derive a screening value for TPA. Although the EPA Integrated Risk Information System (IRIS) database for Dacthal® includes long-term studies on multiple species (EPA 2001), the effects seen for the parent compound may be different in severity or type from those seen for the metabolite.

The mutagenicity data indicate that TPA is not likely to be mutagenic. An *in vivo* carcinogenicity study could be combined with a 2-year chronic study to provide a more definitive answer.

Regulatory Implications

The current federal and state drinking water criteria for Dacthal® are based on toxicological information for the parent compound; however it appears to be the di-acid metabolite, TPA, that occurs in groundwater. Since MDEQ and MDA first confirmed the presence of TPA in Coloma Township, the state has found other sites with the di-acid in the groundwater (C. Rubitschun, MDEQ Water Division, personal communication, 2003; L. Schmelzer, MDA, personal communication, 2003). As the public water supplies in the entire state are tested, it is likely that TPA will be detected in more and more wells. Michigan's economy is based heavily in agriculture and recreation. Dacthal® has been used in both the farming industry and on golf courses. Therefore, groundwater contamination by the metabolite could occur elsewhere in the state.

When contamination of a public drinking water supply is known to exist, MDEQ attempts to abate the contamination. In cases when groundwater cannot be treated, the remedy may be to drill a new well or extend a municipal water supply line to affected areas. Both of these actions are costly ventures to both the communities and the state. Development of more specific screening levels would allow for better regulatory management. If the true contaminant, in this case, is the di-acid metabolite and not the parent compound, and if TPA is determined to be less toxic than Dacthal®, then the drinking water criteria values should reflect these findings. The methods used by the MDA laboratory to verify the presence of TPA in the water samples were experimental in nature. More complex, analytical methods need to be developed and accepted by EPA to detect the mono- and di-acid metabolites of Dacthal® with confidence before regulatory agencies can set a screening level value for the metabolites. If MDEQ, EPA, and ATSDR replace their respective criteria with more appropriate numbers, it is possible that the levels of TPA found in Michigan's groundwater, now or in the future, will not be a public health concern.

ATSDR Child Health Considerations

Children may be at greater risk than adults from exposure to hazardous substances at sites of environmental contamination. A child's lower body weight and higher intake rate results in a greater dose of hazardous substance per unit of body weight. The developing body systems of children can sustain permanent damage if toxic exposures are high enough during critical growth stages. Even before birth, children are forming the body organs they need to last a lifetime. Injury during key periods of growth and development could lead to malformation of organs (teratogenesis), disruption of function, and

premature death. Exposure of the mother could lead to exposure of the fetus, via the placenta, or could affect the fetus through injury or illness sustained by the mother (ATSDR 1998).

The school in which TPA was first detected is now connected to a municipal water supply. Up to 159 homes in Coloma Township that are currently being supplied with bottled water are scheduled to be connected to the municipal supply (C. Rubitschun, MDEQ Water Division, personal communication, 2003). However, until other sites of TPA contamination are identified, children at these sites might be exposed to the chemical in their drinking water. There are no experimental data to indicate what effect exposure to TPA could have on children.

Conclusions

The contamination of groundwater by TPA in Coloma Township, or elsewhere in the state of Michigan, is considered to be an indeterminate public health hazard. A screening level for TPA does not exist, and current toxicological information on the di-acid may not be sufficient to develop a screening level.

Recommendations

1. Establish an appropriate analytical method to detect the mono- and di-acid metabolites of Dacthal®.

2. Determine which chemicals, if any, are present in groundwater samples: Dacthal®, TPA, or the mono-acid.

3. If the regulatory criteria should be for TPA rather than for the parent compound, make appropriate changes to the criteria. This might require more experimental data than currently exist.

Public Health Action Plan

►MDCH and ATSDR will recommend that EPA consider developing an analytical method specific for Dacthal®'s metabolites and establish regulatory criteria as appropriate.

►MDEQ, with MDA's assistance if necessary, will use analytical methods specific for the compounds of interest in future analyses.

►If data indicate that the contaminant is TPA and not Dacthal®, the manufacturer should conduct further toxicological testing to complete the TPA database.

►If data indicate that the contaminant is TPA and not Dacthal®, MDCH will ask MDEQ, EPA, and ATSDR to re-evaluate their respective screening levels and set appropriate criteria for the di-acid.

If any citizen has additional information or health concerns regarding this health consultation, please contact the Michigan Department of Community Health, Environmental and Occupational Epidemiology Division, at 1-800-648-6942.

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Dourson, M. 1994. Methods for establishing oral reference doses. In: Risk Assessment of Essential Elements (Mertz et al., eds.). Washington: ILSI Press. Pp. 51-61.

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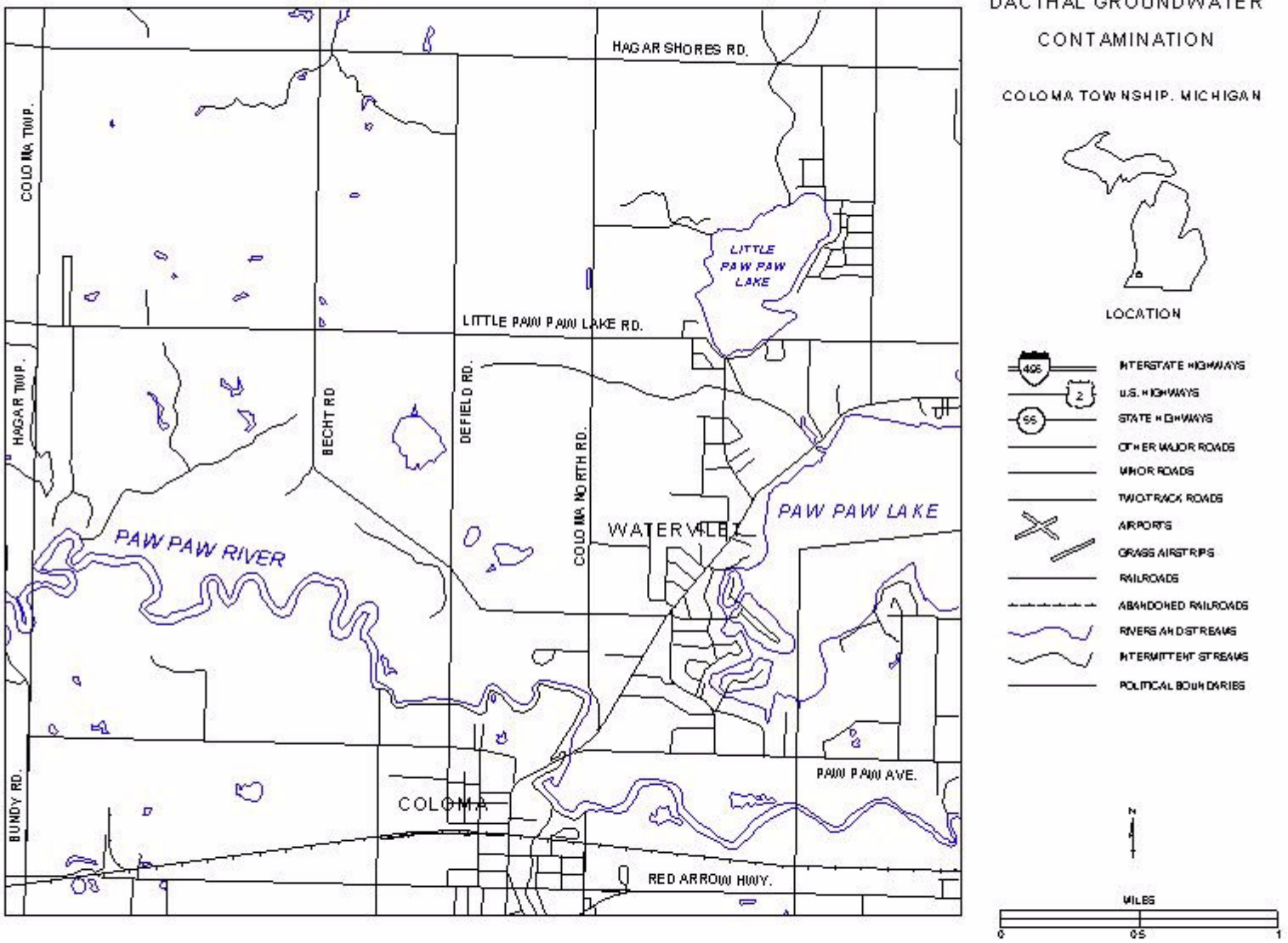
Figure 1.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DEFIELD RD.
COLOMA NORTH RD.
WATERVLIET
PAW PAW LAKE
PAW PAW RIVER
PAW PAW AVE.
BUNDY RD.
COLOMA
RED ARROW HWY.
DACTHAL GROUNDWATER
CONTAMINATION
COLOMA TOWNSHIP, MICHIGAN
LOCATION
INTERSTATE HIGHWAYS
U.S. HIGHWAYS
STATE HIGHWAYS
OTHER MAJOR ROADS
MINOR ROADS
TWO TRACK ROADS
AIRPORTS
GRASS AIRSTRIPS
RAILROADS
ABANDONED RAILROADS
RIVERS AND STREAMS
INTERMITTENT STREAMS
POLITICAL BOUNDARIES
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MILES
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Michigan Department of Community Health
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Appendix A

Evaluation of 2,3,5,6-Tetrachloroterephthalic Acid (Dacthal® Di-Acid) Studies

1. **Hazleton Laboratories Inc. 1961. "28-Day Dietary Feeding – Rats. Material: DAC 1563, DAC 1209, and DAC 876." Letter from R. Weir, Research Application Specialist, dated January 18, 1961 to Diamond Alkali Company. Falls Church, VA.**

Hazleton Laboratories conducted this study for Diamond Alkali Company of Painesville, Ohio, a former manufacturer of Dacthal®. Researchers studied the effects of feeding a 1% dietary level of either the mono-acid (sodium methyl 2,3,5,6-tetrachloroterephthalate) or di-acid metabolite (disodium 2,3,5,6-tetrachloroterephthalate) or a precursor (tetrachloroterephthaloyldichloride) of Dacthal®.

Four groups of male albino Holtzman, Sprague-Dawley rats (10/group) were fed either a control basal laboratory diet or one of the treated diets. The test materials, characterized as fine white powders, were mixed into the lab chow, with the diets being prepared weekly. Feed and water were provided *ad libitum*. Feed consumption, body weight, general physical appearance, and behavior of each rat were recorded weekly. As well, animals were observed for any signs of dermal irritation around the head and forepaws. (The reason for this was not given.)

After 28 days, the animals were sacrificed, and complete autopsies performed on five animals per group. Liver and kidney weights (absolute and relative to body weight) from these animals were recorded. The researchers used Bartlett's test to examine variances for homogeneity, and the F-test (analysis of variance) at $p < 0.05$ to compare the data. The following tissues from these animals were preserved in 10% formalin for possible future histological evaluation: heart, lung, liver, spleen, stomach, small intestine, large intestine, pancreas, kidney, adrenal, testis, bone (sternum), and skin from the area between the eyes and the ears.

During the study, all groups, including the control group, displayed a low incidence of wheezing and nasal discharge. The skin of the head and forepaws of the treated rats did not show signs of irritation, being comparable to controls. There were no statistical differences in body weights or feed consumption between control and treated rats. No deaths of any rats occurred during the study.

Upon necropsy, abscessed lungs were noted in all groups, likely corresponding to the wheezing observed during the study. No gross pathological findings attributable to treatment were reported. There were no statistical differences in terminal body weights or in absolute or relative liver and kidney weights.

Several deficiencies existed in this study, as follows: much detail was missing from the report, such as known purity of the test materials (purity was assumed), the test diets were not assayed to determine actual concentration of test material (mixing a dry test material with a dry diet could lead to homogeneity problems in the resultant mix), it is unclear whether rats were observed daily or only weekly, it is not stated why dermal irritation observations were made, and the data are presented as means, not individually per animal. Additional testing could have been done during this study, such as hematology, clinical chemistry, and urinalysis. More organs should have been weighed. It is not stated whether the tissues preserved in formalin were eventually examined histologically. The statistical power of using only half of the animals per group versus the entire group is weakened. It is understood that Good Laboratory Practices guidelines were not standardized in the early 1960s, which might explain some of the deficiencies of this study.

2. **Skinner, W, and DE Stallard. 1963a. "Dacthal Animal Metabolism Studies." Submitted to the Food and Drug Administration for Partial Fulfillment of Toxicological Data on Dacthal Herbicide. Cleveland: Diamond Alkali Co.**

   Skinner, WA, and DE Stallard. 1963b. "In Vivo Metabolism of Dacthal Herbicide and Disodium 2,3,5,6-Tetrachloroterephthalate." Diamond Alkali Company Report No. DES-63-3002.

   Hazleton Laboratories Inc. 1963. "In Vivo Metabolism Study – Dogs. Material: Pure Dacthal and DAC 1209." Letter from C. Bonfield, Toxicology Department, dated May 13, 1963 to Diamond Alkali Company. Falls Church, VA.

These three reports are believed to be for the same study. This study is a follow-up to a 2-year metabolism study in which dogs (1/sex) were fed 10,000 parts per million (ppm) (1%) Dacthal® in their diet (discussed in EPA's IRIS file for Dacthal®). In that study, analyses of urine and feces showed the majority of Dacthal® being excreted unchanged in the feces. However, the mono- and di-acid metabolites were observed at higher-than-expected concentrations in the excreta, suggesting that the animals hydrolyzed the parent compound first to the mono-acid, then further to the di-acid.

In the current study, 12 dogs (3/group, mixed sexes) were administered 100 or 1,000 milligrams per kilogram (mg/kg) body weight (BW) Dacthal® or 138 or 1,380 mg/kg BW di-acid, via a single oral dose in a capsule. Blood, urine, and feces were collected before dosing, as a control. After dosing, blood and urine were collected at 1, 3, 6, 9, 12, 24, 48, 72, and 96 hours. Feces were collected at 24, 48, 72, and 96 hours post-dosing. Excreta and blood were analyzed for Dacthal®, mono-acid, and di-acid levels. After 96 hours, the dogs were sacrificed and samples of brain, liver, spleen, kidney, skin, bone, muscle, and fat taken. The kidneys, liver, and fat were analyzed for residues of Dacthal® and the mono- and di-acids. Data were tabulated for individual animals.

All dogs appeared healthy and normal during the study except for one female, dosed with 1,380 mg/kg di-acid, that lost 1.4 kg body weight and displayed consistent light brown liquid diarrhea. Upon necropsy, researchers found numerous small hemorrhagic areas in the urinary bladder, congestion of the medullary portion of the kidneys, and a pale brown liver. The other dogs in this dose group appeared grossly normal. Two males dogs dosed with 138 mg/kg di-acid showed pathological changes--one dog showed very small scattered areas of black pigmentation in the lungs, and the other showed congestion in the medullary portion of the kidneys. None of the documents for this study indicated whether these changes might have been treatment-related. It is interesting that two dogs dosed with the di-acid developed what appeared to be kidney problems, the higher-dosed dog having a more advanced case. The pigmentation in the lungs is not likely attributable to treatment.

The analytical results of the excreta from the dogs dosed with Dacthal® indicated that the parent compound was excreted primarily through the feces, whereas the metabolites were excreted primarily through the urine. The results of the blood samples from these dogs showed no true pattern. Only trace amounts of the parent compound were occasionally detected in the blood. Minimal amounts of the di-acid were detected during the first 3 hours after dosing. The mono-acid results showed a dose-response relationship, peaking at 9 to 12 hours post-dosing.

The analytical results of the excreta from the dogs dosed with the di-acid indicated that the majority of the compound was excreted through the feces. About 90% of the total dose was collected in the 24-hour fecal sample, with the remainder of the dose being collected in the urine. Urinary di-acid concentrations peaked within 3 hours after dosing, declined sharply to 12 hours, and then declined at a much slower rate. Blood levels of the di-acid showed a dose-response relationship, peaking the first hour and declining rapidly after that. No mono-acid was reported in the excreta or in the blood.

Trace amounts of Dacthal® were found in the kidney, liver, and fat of the dogs dosed with Dacthal®. Neither metabolite was found in these organs in any dose group except for one dog that received 1,380 mg/kg di-acid (trace of di-acid). The researchers concluded that the chemicals were not stored in these tissues at any significant level.

There are several deficiencies to these reports, as follows: first, it should be verified, if possible, that these three reports are all for the same study; purity of the test materials was not reported; mongrel dogs were used, but purebred laboratory dogs (beagles) would likely be more consistent, between dogs, in their response. There was no indication that the researchers investigated the pathological changes in the kidneys and urinary bladder further to determine if the changes were due to infection or treatment. If the cause for the changes could not be ascertained, then further research would have been warranted. It is not stated whether the other organs taken were weighed or examined histologically. Similar to the rat study discussed previously (Hazleton Laboratories 1961), it is recognized that Good Laboratory Practices guidelines were not standardized in the early 1960s, which might explain some of the deficiencies of this study.

3. **Diamond Shamrock Corporation. 1977. (A 90-day subacute study on male rats; compound in feed. – NO DETAILS.)**

This study is connected to a Diamond Shamrock Corporation report titled "An Evaluation of the Feed and Animal Waste Products from a Toxicity Study in Rats with Tetrachloroterephthalic Acid" (Stallard 1977; not discussed here). The actual report for the study listed here is not obtainable. While this study might be similar to the IRDC study that follows, only male rats (5/group) were used here, whereas 15/sex/group were used in the IRDC study. The doses are the same in both studies.

4. **International Research and Development Corporation (IRDC). 1977. "Ninety Day Toxicity Study in Rats. Compound: DTX 76-0010." Report dated January 3, 1977 to Diamond Shamrock Corporation.**

IRDC conducted this experiment for the Diamond Shamrock Corporation. According to a review of the study by the California Department of Food and Agriculture (CDFA) Medical Toxicology Branch, this study was submitted in response to CDFA concerns about environmental, specifically groundwater, contamination by chlorthal-dimethyl (Dacthal®) (Green and Aldous 1991).

Five groups of Charles River CD rats, 15/sex/group, were fed 0, 50, 500, 1,000, or 10,000 parts per million (ppm) (up to 1%) di-acid in the diet. The test material, characterized as a white powder, was mixed into the lab chow, the diets being prepared weekly. Feed and water were provided *ad libitum*. Feed consumption, body weight, general physical appearance, and behavior of each rat were recorded weekly. Ophthalmoscopic examinations were conducted for all rats before treatment was initiated and at 3 months. Blood and urine samples were taken from 5 rats/sex/group at 1, 2, and 3 months for hematology, biochemistry, and urinalysis.

After 90 days of study, all rats were sacrificed. Selected organs were weighed, and tissues from each rat preserved in 10% formalin. Tissues from the control group and the rats dosed at 10,000 ppm were examined microscopically. The report did not indicate what statistical testing method was used.

All animals appeared and behaved normally during the study. Soft stools or focal areas of alopecia (missing hair) were noted occasionally for a few control and treated rats. Feed consumption values were similar for control and treated rats. Based on feed consumption data, the mean dose per group by the end of the study period was 0, 2.5, 25, 50, and 498 mg/kg/day. Male rats at the 1,000 ppm dosage level gained slightly less body weight when compared to other males in the other groups. This difference was not seen in the 10,000 ppm-dose males. No changes were observed at the 3-month ophthalmoscopic examination. Two male rats at the 50 ppm dosage level and one female at the 10,000 ppm dosage level died following blood collection. No other deaths occurred.

According to the report, no changes in blood or urine parameters appeared to be related to the compound. However, trends seen in these data could have been clarified by

incorporating more data points, that is, including more animals in the sampling. This is discussed further at the end of this evaluation. The researchers did sample from the same animals each time, which presents a more accurate picture of changes over time than if they had sampled randomly.

According to the report, no compound-related gross pathologic lesions nor organ weight variations were observed upon necropsy, nor did microscopic examination of the tissues from the control and 10,000 ppm-dose groups show any non-spontaneous lesions. However, trends seen in the histological data could have been clarified by incorporation of more data points, specifically by including the other dose groups.

A key deficiency in this study is that the researchers limited the data evaluated. Although not statistically significant, there was a noticeable decline in blood glucose levels in the male rats in the 1,000 ppm dose group and in both sexes in the 10,000 ppm dose group. If 10 or all 15 animals/sex/group had been sampled, statistical power would have increased, and it could have been determined if this finding, or any questionable trend, was an artifact or treatment related. Additionally, by not evaluating the tissues from the other dose groups, it cannot be determined if a dose-response relationship was present. Other deficiencies in this study include not knowing the purity of the test material and not analyzing the diets for content or homogeneity. It is recognized that Good Laboratory Practices guidelines were only just beginning to be standardized in the mid-1970s, which might explain some of the deficiencies of this study. This report, however, did provide individual animal data and more detail on study protocol than did earlier reports.

5. **Barfknecht, TR. 1984. "DNA Repair Test in Rat Hepatocyte Primary Cultures with Tetrachloroterephthalic Acid." Waverly, PA: Pharmakon Research International Inc. Doc. No. 666-5TX-84-0042-002.**

The following is a summary of the study, provided by CDFA: The di-acid, 99% purity, was assayed *in vitro* in the unscheduled DNA synthesis test. Male Fischer 344 rat heptocytes were used in triplicate wells at untreated, 0 (ethanol), 20, 60, 200, 600, 2,000, and 6,000 micrograms (μg) per well. Positive control was 2-AAF (2-acetaminofluorene). No increase in unscheduled DNA synthesis was indicated (Green and Gee 1990d).

6. **Godek, EG. 1984. "Salmonella/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames Test) with Tetrachloroterephthalic Acid." Waverly, PA: Pharmakon Research International Inc. Doc. No. 666-5TX-84-0061-002.**

The following is a summary of the study, provided by CDFA: The di-acid, 99% purity, was assayed *in vitro* in a reversion assay (the Ames test). *Salmonella typhimurium* strains TA98, TA100, TA1537, and TA1538 were used in triplicate plates in the presence and absence of activation (Aroclor 1254-induced Sprague Dawley rat liver fraction) at 0 (95% ethanol), 50, 150, 500, and 1,500 μg per plate. No increase in the reversion frequency was indicated (Green and Gee 1990a).

7. **Godek, EG. 1985. "Mammalian Cell Forward Mutation Assay in the CHO/HGPRT System with Tetrachloroterephthalic Acid." Waverly, PA: Pharmakon Research International Inc. Doc. No. 666-5TX-84-0072-0002.**

The following is a summary of the study, provided by CDFA: The di-acid, 99% purity, was assayed *in vitro* in a forward mutation test (5-hour exposure). Chinese hamster ovary cells (clone K1, subclone BH4) were used in duplicate cultures in the presence and absence of activation (Aroclor 1254 induced male Sprague-Dawley rat liver homogenate) at 0 (95% ethanol), 100, 500, 1,000, 1,500, and 2,000 micrograms per milliliter (µg/mL). Seven-day expression time was followed by mutant selection with thioguanine. No increase in forward mutation frequency was indicated (Green and Gee 1990b).

8. **Major et al. 1985. "A 30-Day Oral Intubation Study in Rats with Tetrachloroterephthalic Acid (SDS-954)." Painesville, OH: SDS Biotech Corporation. Doc. No. 665-5TX-84-0007-001.**

This study was conducted to supplement the findings of the 90-day subchronic study discussed previously (IRDC 1977). The earlier study had been prompted by concerns of groundwater contamination by the di-acid in Long Island, New York, likely in Suffolk County as reported in the Reregistration Eligibility Decision document by EPA (EPA 1998).

Four groups of CD (Sprague-Dawley) rats, 10/sex/group, received an oral dose, via intubation, of 0, 100, 500, or 2,000 mg/kg/day of the di-acid. The vehicle was 0.5% methylcellulose in distilled water. Dose volume was 10 mL/kg. According to the report, pre-study testing with mixes of test material in rat diet indicated that an acceptable method for diet assay could not be developed. Oral intubation was selected as the route because, according to the report, a suitable method for analysis of test material suspensions was developed. The test material was not tested in rats via drinking water (which would be the likely human route of exposure) because the desired dose levels to be tested exceeded the solubility of the test material in water. (The reported solubility was 0.5 g/100 ml.)

During the study, rats received food and water *ad libitum* and were observed for general appearance and behavior twice daily after dosing. Complete physical examinations were conducted and feed consumption recorded weekly. Body weights were measured on Days 3 and 7 of each week of the study. Hematology, clinical chemistry, and urinalysis were performed after the 30-day feeding period, 4-5 days before necropsy of the animal. The cause for delay between the last dose and the necropsies was not discussed in the report.

Necropsies were performed on all animals. Researchers recorded the weights of the adrenal glands, brain, gonads, heart, liver and kidneys from all animals. Tissues from all animals were taken and preserved in 10% formalin, with the full array of samples from the control group and 2,000 mg/kg/day-dose group being examined histologically. Selected tissues from the lower-dose groups were also examined microscopically.

Researchers used Dunnett's multiple comparison test and analysis of variance to compare the data.

Analysis of the test material suspensions showed a homogeneous mix and near 1:1 (96-102%) actual-to-theoretical concentrations. Purity of the di-acid was >99%.

Body weight and feed consumption data showed no compound-related differences between treatment groups. Two rats died during the study. One control male rat died during Week 4 as a result of a dosing accident. One male rat in the 2,000 mg/kg/day-dose group died during the last week of the study, before the scheduled necropsy. Gross pathology of this rat showed pale kidneys; dilated renal pelvis; and firm, dark red prostate and seminal vesicles. Histology results correlated with the gross findings. The pathologist's report indicated that this was likely an acute genitourinary tract infection, unrelated to compound administration; however there was no further testing to verify that diagnosis.

The rats in the high-dose (2,000 mg/kg/day) group were noted to have soft stools throughout the study, with the incidence being greater during the first and last weeks. The stools were formed pellets that were soft in consistency. The researchers concluded, based on the rest of the data collected, that this finding was the result of either an increase in gastrointestinal tract motility or a large amount of test material in the gastrointestinal tract, and subsequent interference with water reabsorption in the large intestine. This is a plausible explanation, and although the finding is compound- and dose-related, toxicological significance is questionable.

The results from the hematology, clinical chemistry, and urinalysis showed some statistically significant differences between control group rats and treated group rats; however the researchers believed that these were not biologically significant because the findings fell in the range of historical control data for CD rats. However, what the researchers failed to mention was that, in the 2,000 mg/kg/day-dose males, the mean glucose level was statistically significant less than the control, and a similar finding was seen in the 90-day study discussed earlier (IRDC 1977). In the current study, the high-dose males also had lower mean albumin and potassium levels than controls. These findings might be related to the incidence of soft stools for both sexes in this group.

Organ weights (absolute and relative to body or brain weight) showed no statistically significant differences between groups. Any histological changes were considered to be spontaneous and not related to compound administration.

Both the procedure and the report for this study are a marked improvement from the earlier studies discussed previously. Details are described well, and the data tables allow reviewers to look at raw data. One deficiency is the lack of discussion regarding the lower serum glucose levels in high-dose males and the similar finding in the IRDC (1977) study. Because there were no other corresponding changes, other than soft stool in both sexes, in the current study, it may be that this finding was incidental or a treatment

effect of inconsequential importance. Another deficiency is that the diagnosis of an acute genitourinary tract infection in one of the male rats that died was not verified.

9. **Mizens et al. 1985. "A Teratology Study in Rats with Tetrachloroterephthalic Acid (SDS-954)." Painesville, OH: SDS Biotech Corporation. Doc. No. 687-5TX-84-0035-002.**

This study was conducted by Argus Research Laboratories in Horsham, Pennsylvania, for SDS Biotech Corporation. Four groups of pregnant Charles River Crl:CoBS® CD® rats, 25/group, were dosed via oral intubation with 0, 625, 1,250, or 2,500 mg/kg/day of the di-acid on Gestation Days (GD) 6-15. (Doses were chosen on the basis of a previous study done by Argus. Details of that study were not given.) The vehicle was 0.5% methylcellulose. Dosing suspensions were prepared twice during the dose period.

The rats were observed at least twice daily for general appearance and behavior. Body weights were recorded on GD 0 and daily beginning at GD 6 through study termination. Feed consumption was recorded in intervals, from GD 0-6, then in 3-day intervals through study termination.

On GD 20, the rats were sacrificed. The ovaries were examined for number and placement of corpora lutea. The uterus was examined for number and placements of implantations, live and dead fetuses, and early and late resorptions. Tissues from the dams were retained for potential histological examination. Live fetuses were killed and examined for soft tissue anomalies and skeletal variations.

Data were analyzed using Bartlett's test for parametric values and Kruskal-Wallis's test for nonparametric values. The data were also tested for homogeneity, and variance and covariance were analyzed.

The 2-week acclimation period that preceded mating was extended by 1 week due to clinical signs of a sialodacryoadenitis (SDA) viral infection at the end of the 2 weeks. (SDA is a respiratory and digestive system viral disease of the rodent, affecting the salivary and lacrimal glands. Clinical symptoms seen in the rats in this study included enlarged cervical glands, red tearing of the eyes, and relaxed eyelids.) An Argus veterinarian judged the infection to have cleared before the females were mated.

Body weights did not vary significantly between groups. On GD 6-9, the high-dose (2,500 mg/kg/day) females experienced a significantly reduced feed consumption rate, which then rebounded significantly after dosing stopped. The researchers concluded this was probably an adjustment to the test material administration. There were no deaths during the study.

Physical observations attributed to test material administration were soft or liquid feces, red-colored fecal mucus, and reddened anal region in the high-dose group. As discussed in Major et al. (1985), the toxicological significance of this finding is questionable. A statistically significant increase in excess salivation was observed in the 1,250 and 2,500

mg/kg/day-dose groups. The observation was considered to be a dose-related compound effect and reportedly related to the acidity of the test material. However, there were no pH values given for the suspensions in this document or related reports (Karrenbrock 1985a, b).

At least 22 rats/group were pregnant at the termination of the study. Necropsy of the dams did not reveal any lesions that could be attributed to the administration of the test material. Examination of the ovaries and uteri did not show any biologically or statistically significant differences between groups. Fetal weight and sex distribution were not affected by treatment. Variations seen at the gross, visceral, and skeletal levels were considered spontaneous and not treatment related. The highest incidence of fetal variations was observed in the control group and the lowest in the high-dose group.

As discussed for Major et al. (1985), both the procedures and the report for this study are a marked improvement over earlier studies.

10. SanSebastian, JR. 1985. "In Vitro Sister Chromatid Exchange Assay in Chinese Hamster Ovary Cells with Tetrachloroterephthalic Acid." Waverly, PA: Pharmakon Research International Inc. Doc. No. 666-5TX-84-0062-002.

The following is a summary of the study, provided by CDFA: The di-acid, 99% purity, was tested *in vitro* in a sister chromatid exchange assay (5-hour exposure). Chinese hamster ovary cells (CHO-K1-BH4) were used in duplicate cultures in the presence and absence of activation (Aroclor 1254 induced male Sprague-Dawley rat liver fraction) at 0 (1% DMSO), 200, 500, 1,000, 1,500, and 2,000 μg/mL. The pH of the test incubation medium was lowered to "acidic" upon addition of the ≥ 1000 μg/mL, limiting the highest practical concentration. No increase in sister chromatid exhange frequency was indicated (Green and Gee 1990c).

11. Siou, G. 1985. "The Micronucleus Test in Mice with Tetrachloroterephthalic Acid (SDS-954)." Versailles, France: Experimental Cytology and Research in Industrial Toxicology, Histopathology Laboratory. Doc. No. 666-5TX-84-0071-002.

The following is a summary of the study, provided by CDFA: The di-acid, 99% purity, was tested *in vivo* in a bone marrow cytogenetics micronucleus assay. Swiss mice, 7/sex/group/sampling time, were used. A single dose of 0, 1,000, 5,000, or 10,000 (males) or 0, 500, 2,500, or 5,000 (females) mg/kg was administered by gavage. (A preliminary test indicated females were more sensitive to the toxic effects than were males.) The vehicle was 0.5% Methocel E15 Premium. The polychromatic/normochromatic ratio of erythrocytes indicated that bone marrow toxicity occurred at 48 and 72 hours in males at the high dose and at 48 hours in females at the high dose. An increase in the frequency of micronucleated polychromatic erythrocytes in males at the 48-hour sampling was indicated, suggesting a weak clastogenic potential. However, that dose was twice the recommended maximum dose according to Toxic Substance Control Guidelines and was into the range which elicited bone marrow

toxicity. Therefore, the finding did not indicate appreciable concern (Green and Gee 1990e).

Appendix A References

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Certification

This *Dacthal® Groundwater Contamination – Additional Toxicological Data Health Consultation* was prepared by the Michigan Department of Community Health under a cooperative agreement with the Agency for Toxic Substances and Disease Registry (ATSDR), an agency within the U.S. Department of Health and Human Services. It is in accordance with approved methodology and procedures existing at the time the health consultation was begun.

Technical Project Officer, SPS, SSAB, DHAC, ATSDR

The Division of Health Assessment and Consultation, ATSDR, has reviewed this public health consultation and concurs with the findings.

Chief, State Programs Section, SSAB, DHAC, ATSDR