A REVIEW OF THE GENERAL ACUTE TOXICITY STUDIES OF *BORON #10*®

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ABSTRACT

A series of animal studies was conducted to assess the acute toxicity of *Boron #10®*. The tests conducted were the following: acute oral toxicity in rats, acute dermal toxicity in rabbits, primary dermal toxicity in rabbits, primary eye irritation in rabbits, dermal sensitization in guinea pigs, and 4-hour inhalation toxicity in rats. The principal results were the following:

- the oral LD_{50} of *Boron #10®* was calculated to be 3,765 (mg/kg) in male rats;
- no adverse effects were noted for acute dermal exposure to the product in rabbits, and the dermal LD_{50} was considered to be greater than 2,000 (mg/kg) of body weight;
- minimal and reversible skin irritation was observed when 500 milligrams of product was applied to the skin of rabbits;
- mild and reversible irritation to the eye was observed when 100 milligrams of the product was placed in the eye of rabbits;
- there was no evidence of contact dermal sensitization in Guinea pigs; and
- the LC_{50} was considered to be greater than 5.8 milligrams per liter of air.

Additionally, *Boron #10®* is to be applied only to “cracks and crevices” (i.e., areas that are not commonly accessed), per its labeling. This application would likely constitute only about 13-19% of a broadcast application. Such broadcast applications of *Boron #10®* have been studied, however, and even a broadcast application using worst-case conditions did not generate a human exposure of health concern. These results indicate that unless a great quantity of *Boron #10®* is ingested, which is unlikely with the labeled use of this product, no adverse health effects would be expected.

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INTRODUCTION

INTERTOX, INC. has extensive experience reviewing the general use and toxicity of boron-based products, and sodium polyborate in particular, the chemical name of *Boron #10*®. A general review of boron-based compounds indicates that these compounds occur naturally in low concentrations in air, water, and soil and are of generally low toxicity. Boron is an essential micronutrient for normal growth in plants. Additionally, boron-containing compounds have a variety of agricultural and non-agricultural uses, including applications in residential, commercial, medical, veterinary, industrial, forestry, and food handling settings.[1, 2] It is estimated that the average person ingests between 10 and 25 milligrams of boron-containing compounds daily.[3] Of all the agricultural uses, a small percentages of boron-based compounds are used as insecticides and are particularly effective in killing fleas, ants, spiders and cockroaches.[4]

This paper evaluates the general acute toxicity of *Boron #10*®, a commercial pest-control product, based on the results of six animal toxicity studies of the product. The studies indicate that the effects of exposures to *Boron #10*® are minor unless large doses are taken orally. Compounds like *Zone Defense*® with a benchmark toxicity measure known as LD_{50} in excess of several grams per kilogram of body weight (in the same range as table salt), can be classified as "relatively harmless."[5]

ANIMAL TOXICITY STUDY OVERVIEW

Animal toxicity studies are performed to indicate whether a given compound is likely to be toxic to humans. For each study, test animals are exposed to high dosages of the test product and both behavioral and physical responses are studied. The species chosen for each type of study is chosen because of its demonstrated sensitivity to a given type of exposure (e.g., New Zealand White Albino rabbits are generally very sensitive to substances applied to the skin) and because of knowledge that the animal's biological means of processing such exposures is most likely similar to that of humans.

Given the difficulty and expense of conducting studies of long-term exposures, short-term, or "acute," toxicity studies are the most commonly performed. The principles described above apply to acute toxicity studies. In such studies, dosages are often extreme in order to produce obvious, observable effects. One of the most commonly measured observed effects of such an extreme exposure is death of the test animal. Survival rates in these extreme exposures are used to estimate a toxicity measure known as the "lethal-dose-50," or LD_{50}, defined as the dose of a compound that would be lethal for 50% of a group of test animals. Because individual test animals have differing capacities to survive exposure to a given compound, some being highly sensitive to exposure and some being relatively insensitive, this measure of lethality for half of the test group has become a standard measure of acute toxicity. Additionally, because a compound can elicit a toxic response through some routes of exposure and not others, acute toxic response to *Boron #10*® was studied through skin and eye exposures and through ingestion and inhalation.

[1] Goldberg, 1993.
[2] US EPA, 1993.
[3] US EPA, 1993.
[4] US EPA, 1993.
[5] Klaassen, 1996.

To simplify application of toxicity values to humans, LD_{50}s are measured in mass of compound per mass of body weight, e.g., milligrams *Boron #10®* per kilogram body weight (mg/kg) of the subject. LD_{50} values have been determined for many compounds. Therefore, given the results of the studies reviewed here, toxicity comparisons can be made between *Boron #10®* and many other products and compounds using this value. The animal studies reviewed here were conducted by American Biogenics Corporation in Decatur, Illinois, in 1986. They were performed in conformity with the Food and Drug Administration (FDA) and Environmental Protection Agency (EPA) Good Laboratory Practice regulations, which require, among other practices, the humane treatment of animals during testing. The six studies are reviewed and summarized in the following subsections to evaluate the potential for adverse health effects in humans.

ACUTE ORAL TOXICITY[6]

Boron #10® was tested for acute oral toxicity using groups of five male and five female rats. Groups of animals were tested at the following dosages of *Boron #10®*: 2,818, 3,548, 3,758, 3,981, and 4467 mg/kg.[7] The oral LD_{50} was calculated to be 3,130, 3,765, and 3,339 (mg/kg) for females, males, and for combined sexes, respectively.[8] Subjects that died, did so between days one and two of the study. As would be expected, one consistent effect of high oral doses of *Boron #10®* toxicity was weight loss. Several other clinical manifestations were noted for some animals over the course of the experiment and during postmortem inspection.[9]

ACUTE DERMAL TOXICITY[10]

Boron #10® was tested for acute dermal toxicity in New Zealand White Albino rabbits (five males and five females). Fur from the back and side of each subject's trunk (approximately ten percent of the surface of the rabbit) was clipped. Rabbits were exposed to 2,000 milligrams of *Boron #10®* per kilogram of body weight as a dose. The product was held in place on the clipped section of the animal's body with gauze and then the entire trunk of the animal was covered with an impervious

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6 The purposes of an acute oral toxicity test are to 1) estimate acute toxicity, 2) identify organs that are affected by compound exposure and identify the nature of those toxic effects, 3) determine the reversibility of the toxic effects, and 4) provide a dose range for further studies, if necessary. In this test, as in most tests of this type, the test animals were rats.

7 The first group was tested at the dosage of 5,000 (mg/kg) and all test animals died. The doses were lowered as reported.

8 Smith et al., 1986b.

9 These included: crusty muzzle, lethargy, loose stools, muscle tremors, sensitivity to touch, crusty eyes, and inability
to coordinate voluntary muscular movements, slow respirations, yellow/brown stained fur around the anal region, salivation, exhaustion, irregular breathing, lacrimation, squinting, and/or death. Examination of the animals that died during the experiment revealed the following: red discolorations of the lungs and thymus; distention of the stomach and intestines with fluid; abrasion on scrotum; pale small intestine; red discoloration of the heart; pale discoloration and red mottling of the liver; and dilated pelvis of kidneys.

10 The purposes of an acute dermal toxicity test are to 1) determine if exposure to a large dose of a compound can result in absorption through the skin in quantities great enough to result in an acute toxic effect, 2) determine the reversibility of the toxic effects, and 3) provide a dose range for further studies, if necessary. For those compounds that cross the skin in sufficient quantities and cause death, a dermal LD_{50} can be calculated.

binder[11] to prevent the product from migrating from the site. These rabbits were exposed for a full 24-hour period because there were no initial adverse effects. Following the exposure period, the site was wiped and the rabbits were monitored for adverse health effects. The only signs noted during the study were a slight redness and swelling of the test site on three of five males and three of five females. These signs returned to normal within two days after *Boron #10®* was removed. Since there were no adverse health effects or deaths, the authors conclude that "the acute LD_{50} was considered to be greater than 2 grams per kilogram of body weight."[12]

PRIMARY DERMAL TOXICITY[13]

Boron #10® was tested for primary dermal toxicity in six male New Zealand White Albino rabbits. A 2.5-centimeter square of fur on the back of the rabbits was clipped, and 500 milligrams of *Zone Defense®* was applied with a gauze wrapping. The entire trunk was wrapped in an impervious binder. The treatment was removed after a 4-hour exposure period. The rabbits were then examined on a daily basis for adverse effects. Three rabbits showed immediate but slight swelling when the gauze was removed from the skin. All skin changes disappeared within 24 hours after removal of the product. The Primary Irritation Score was 0.3 on the 0 to 8 scale. Under this exposure scenario, the investigators judged exposure to *Boron #10®* to be "minimally irritating."[14]

PRIMARY EYE IRRITATION[15]

A group of six female adult New Zealand White Albino rabbits was used in this study. A dose of 100 milligrams of *Boron #10®* was placed onto the everted lower lid of the right eye on each animal. The upper and lower lids were then held together for one second to prevent the loss of any of the test agent. The left eye was not treated and served as the control so that comparisons could be made. The eyes were then examined 1, 24, 48, and 72 hours after the test agent was administered. One hour after treatment, inflammation of the iris and noticeable discharge (no details reported of the type of discharge) with moistening of the lids and area around the eye was observed.

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11 The impervious binder is usually a plastic wrap, adhesive tape and masking tape.
12 Smith et al., 1986a.
13 The purposes of a primary dermal toxicity test are to 1) determine if exposure to a large dose of a compound can irritate skin, 2) determine the reversibility of the irritation, and 3) provide a dose range for further studies, if necessary. In this test, as in most tests of this type, test animals were New Zealand White Albino rabbits and their exposure to the test product was for 4 hours. Substances that irritate skin may cause the following signs: none, reddening, swelling, and, in some cases, the development of lesions. A scale, called the Primary Irritation Scale, ranging from 0 (no effects) to 8 (most severe), is used to measure dermal irritation.
14 Smith et al., 1986d.
15 Primary eye irritation studies are conducted to 1) observe the effect that large doses of a compound have on the eyes and body of a rabbit, 2) determine the reversibility of the toxic effects, and 3) provide a dose range for further studies, if necessary. In this test, as in most tests of this type, test animals were New Zealand White Albino rabbits. Health effects can range from no effect or reversible adverse effects (e.g., redness of eye structures, swollen iris) to irreversible damage to the eye (e.g., clouding of the cornea, corrosive action to the tissues). The Primary Eye Irritation Scale is a rating system used to measure the degree of severity of a chemical exposure. The grading system is based on the effects of a test compound on three eye structures: 1) the cornea (the transparent structure that covers the iris and pupil), 2) the iris (which surrounds the pupil), and 3) the conjunctivae (the white of the eye). The scale ranges from 0 (no effect for any of the three structures) to 110 (severe effect in all three structures).

These signs had disappeared in all but two rabbits by the 24-hour examination period. The two rabbits with minimal but observable signs at 24 hours were normal at the 48-hour exam. According to the investigators, "*Zone Defense*® was considered to be mildly irritating."[16]

DERMAL SENSITIZATION[17]

Boron #10® was tested for dermal sensitization on 20 guinea pigs. Of these, ten were assigned to the test group and received nine, 500-milligram applications of *Boron #10*® on 4 x 4-centimeter Webril patches attached to the animal with hypoallergenic tape.[18] The ten other guinea pigs served as a control group that remained untreated. Subjects were given treatments three times a week for three weeks. Two weeks after the ninth application, all animals in the two groups received a single, 500-milligram application of *Boron #10*®. The entire trunk of the animal was wrapped in an impervious binder. After a 6-hour exposure period, the binders and patches were removed and the skin evaluated. The exposure sites on the animals were assessed for redness or swelling of the skin, lesions and other dermal reactions. The results show no signs of skin sensitization at any point during the study. The investigators report that "*Boron #10*® was not considered to be a contact dermal sensitizer."[19]

FOUR-HOUR INHALATION[20]

Ten young adult albino rats, five males and five females, were used to determine the acute inhalation toxicity of *Boron #10*®. The subjects received a 4-hour, whole-body inhalation exposure to *Zone Defense*®, released into the test atmosphere as fine airborne particles. The average airborne gravimetric concentration was calculated to be 5.8 milligrams of *Boron #10*® per liter of air. The animals were removed from the chamber and evaluated for 14 days for signs of adverse health effects.

No animals died during exposure or during evaluation, so an LC_{50} (lethal concentration in air for 50 percent of a test group) could not be established near the tested concentration. Removing the animals from the chamber, investigators only noted the following: poor coat quality and crusty eyes, nose,

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16 Smith et al., 1986e.

17 The purpose of a dermal sensitization study is to determine the potential of a compound to sensitize skin. Sensitization is also called hypersensitivity or an allergy. Sensitization is a complex biochemical phenomenon in which the body requires a previous exposure to elicit a reaction. In this test, as in most tests of this type, the test animal was the guinea pig and multiple treatments of the test product were applied to the shaven skin of guinea pigs over a period of 2 to 4 weeks. The test material was applied again 2 to 3 weeks after the last treatment and the skin was scored for swelling and redness. This last treatment is a low, non-irritating dose. The skin is graded on a scale of 0 (no effects) to 4 (severe effects).

18 Two guinea pigs were used for the preliminary range-finding trials. Subjects were given either 15, 50, 150, or 500 milligrams of *Boron #10*® mixed in 0.3 milliliters of water (3, 10, 30, or 100 percent solutions). Each animal was tested with two of these dosages on separate 4 x 4-centimeter Webril patches. There were no observable dermal reactions for any dose, so the 500 milligram solution was used for the rest of the experiment.

19 Smith et al., 1986c.

20 The purpose of a 4-hour inhalation study is to determine if breathing air with a high concentration of *Zone Defense*® can adversely affect the body or the respiratory system of an animal. Rats are continuously exposed for 4 hours to a compound dispersed in the atmosphere of a special exposure chamber. The effects can range from no adverse effects to death of the animal. If death occurs, an LC_{50} can be calculated.

and muzzle. The investigators did not specify whether these results were due to *Boron #10*® specifically or due to a general dust exposure. The only adverse health signs observed during the 14-day, post-exposure period were crusty muzzles for two animals on day one. After 14 days, the subjects euthanized. Examination of their bodies revealed that of all the subjects exposed, only one female subject had an abnormality: an enlarged but otherwise normal renal pelvis (an anatomical structure that funnels droplets of urine from the kidneys to the urinary bladder) in its right kidney. It could not be determined from the investigator's report whether they believed that the dilated renal pelvis was a result of *Boron #10*® exposure or an individual variation of the animal (i.e., birth defect). The report concluded that, "under the conditions tested, an LC_{50} for *Boron #10*® would be greater than a gravimetric concentration of 5.8 milligrams per liter of air."[21]

OVERVIEW AND CONCLUSION

Boron compounds exist naturally throughout our environment and boron is an element commonly ingested in small amounts by humans. Boron and its naturally occurring compounds generally have low toxicity and pose little threat to human health unless great quantities are ingested or cross damaged skin. This appears to hold true for the boron-containing pesticide, *Boron #10*®. The six animal studies reviewed herein provide fundamental information needed for assessing the inherent acute toxicity of *Boron #10*®. The studies appear to have been performed using appropriate experimental protocols. The results of the reviewed studies indicate that the effects of exposures to *Boron #10*® are minor and temporary unless large doses are taken orally. Specifically, these studies show that *Boron #10*®*:*

- has an estimated oral LD_{50} of 3,765 mg/kg;
- has a dermal LD_{50} of more than 2,000 mg/kg;
- is a minimal dermal irritant;
- is mildly irritating to the eyes;
- is not a contact dermal sensitizer; and
- has an LC_{50} of greater than 5.8 mg/l.

These studies focus on acute toxicity. Long-term, or "chronic," toxicity is not directly addressed. However, given that related compounds, such as boric acid, have shown evidence of noncarcinogenicity and have not shown evidence of other genetic toxicity, it is likely that *Boron #10*® also is not toxic in those ways.[22] This is further evidenced in that boric acid itself is believed to be responsible for the toxic effects of boron-containing compounds.[23] In practice, this means that even a prolonged exposure of several years would not result in tumor development. Per its labeling, *Boron #10*® is to be administered to "cracks and crevices" (i.e., areas that are not commonly accessed), as opposed to "broadcast" application as is typical of spray-applied pesticide "foggers." An earlier study determined, however, that even if *Boron #10*® were applied in a broadcast manner, using worst-case conditions, no adverse health effects would be expected from exposures related to

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[21] Newton et al., 1986.
[22] US EPA, 1993.
[23] ERM-ETI, 1994.

the application.[24] This study determined that ingestion, as opposed to dermal contact or inhalation, would be the dominant route of exposure for such a product. In a typical room, the amount of *Boron #10*® applied in cracks and crevices would constitute only about 13-19% of the amount applied in a broadcast application.[25] Additionally, the *Boron #10*® that is applied to cracks and crevices would be far less accessible to human contact, especially ingestion, than would be most of the pesticide applied in a broadcast manner. Therefore, the exposure to *Boron #10*® resulting from a crack and crevice application would be substantially smaller than even the negligible exposure resulting from the broadcast application considered in the study. Based on this information and the information obtained from the animal studies, we would not expect adverse human health effects to result when *Boron #10*® is used as labeled and directed.

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[24] ERM-ETI, 1994. The purpose of ERM-ETI's dosimetry study was to estimate the amount of a product of identical composition to *Boron #10*® that could be absorbed across intact skin. Different styles of carpet were treated with application rates (3.8 and 9.1 grams per square foot) higher than the labeled rate for the identical product and similar to those for *Boron #10*®. Percale material was used as a surrogate human skin and pressed into treated carpets by a 25-pound device called a California Roller. Exposure to the powder was assessed by measuring the boron content of the percale and transforming the values into boric-acid equivalents. The amount of boric acid was calculated based on several exposure scenarios, including a worst-case scenario (the highest application rate, total skin exposure of a child or adult body, and transfer of 1 percent of the boron across skin). Using this scenario, it was estimated that a child and an adult would be exposed to 2.2 milligrams and 5.4 milligrams of boric acid per exposure, respectively.

[25] This estimate is based on the ratio of a 6"-wide perimeter to total room area, rooms ranging from 10' x 10' to 15' x 15', and assuming that "crack and crevice" application would be only to the perimeter of the room. The larger the room, the smaller the ratio. INTERTOX performed an extensive, although not exhaustive, literature search related to "crack and crevice" application of pesticides. The on-line databases searched included DIALOG® ALLSCIENCE, the Federal Register, the Code of Federal Regulations, and the EPA Web Server. INTERTOX also contacted the Office of Pesticide Programs of the EPA for additional information. The available literature that focused on differentiating "crack and crevice" and broadcast application techniques did not provide information regarding comparative expected exposures to particulate pesticides. Rather, literature obtained focused solely on volatiles. That information is not appropriate for extrapolation to particulates.

REFERENCES

Draize JH, Kelley EA. (1959) The urinary excretion of boric acid preparations following oral administration and topical applications to intact and damaged skin of rabbits. *Toxicol Appl Pharmacol* 1:267-276.

ERM-ETI. (1994) Dosimetry Study for *Boron #10.* ERM-Environmental Toxicology International, Inc. Seattle, WA November 12.

Garabrant DH, Bernstein L, Peters JM, Smith TJ. (1984) Respiratory and eye irritation from boron oxide and boric acid dusts. *J Occup Med* 26:584-586.

Goldbloom RB, Goldbloom A. (1953) Boric acid poisoning: report of four cases and a review of 109 cases from the world literature. *J Pediatr* 43:631-643.

Gosselin RE, Smith RP, Hodge HC, Braddock JE. (1984) In: 5th ed. *Clinical Toxicology of Commercial Products,* Baltimore: Williams and Wilkins, 69.

Goldberg S. (1993) Chemistry and mineralogy of boron in soils. In: Gupta UC, ed. *Boron and Its Role in Crop Production*. Boca Raton: CRC Press, 3-44.

Heindel JJ, Price CJ, Field EA, Marr MC, Myers CB, Morrissey RE, Schwetz BA. (1992) Developmental toxicity of boric acid in mice and rats. *Fund Appl Toxicol* 18:266-277.

Klaassen C. (1996) *Casarett and Doull's Toxicology*, New York: McGraw-Hill and Company.
Newton PE, Horath LL, Malone TC, Kush J, Becker SV, Mackellar DG. (1986) Four Hour Acute Aerosol Inhalation Toxicity Study in Rats of Boron #10®. American Biogenics Corporation, Decatur, Ill.

Pfeiffer CC, Hallman LF, Gersh I. (1945) Boric acid ointment: a study of possible intoxication in the treatment of burns. *JAMA* 128:266-274.

Pfeiffer CC, Jenney EH. (1950) The pharmacology of boric acid and boron compounds. *Bull Natl Formul Comm* 18:57-80.

Siegal E, Wason S. (1986) Boric acid toxicity. *Ped Clin North Amer* 33:363-367.

Shaheen L. (1992) Boric acid stops flea hopping - naturally. *Pest Control* 60(6): 50-51.10

Smith SH, Kreuger JC, Kush J, Becker SV Mayhew DA, MacKellar DG. (1986) Acute Dermal Toxicity Study in Rabbits with *Boron #10®.* American Biogenics Corporation, Decatur, Ill.

Smith SH, Kreuger JC. (1986) Acute Oral Toxicity Study in Rats with *Zone Defense®.* American Biogenics Corporation, Decatur, Ill.

Smith SH, Mellon KA, Kush J, Mayhew DA, Skelley A. (1986) Dermal Sensitization Study in Guinea Pigs with *Boron #10®.* American Biogenics Corporation, Decatur, Ill.

Smith SH, Kreuger JC, Kush J, Mayhew DA, MacKellar DG. (1986) Primary Dermal Irritation Study in Rabbits with *Boron #10®.* American Biogenics Corporation, Decatur, Ill.

Smith SH, Kreuger JC, Kush J, Mayhew DA, and MacKeller DG. (1986) Primary Eye Irritation Study in Rabbits with *Boron #10®.* American Biogenics Corporation, Decatur, Ill.

U.S. EPA. (1993) Reregistration eligibility decision (RED). Boric acid and its sodium salts. U.S. EPA 738-R-93-017. U.S. Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances, Washington D.C.

Whorton D, Haas J, Trent L. (1992) Reproductive Effects of Inorganic Borates on Male Employees: Birth Rate Assessment. Final Report for U.S. Borax & Chemical Corporation. ENSR Sciences, Alameda. CA.

Wong LC, Heimbach MD, Truscott DR, Duncan BD. (1964) Boric acid poisoning: report of 11 cases. *Canad Med Assoc J* 90:1018-1023.