LINDA LINGLE
Governor

SANDRA LEE KUNIMOTO
Chairperson, Board of Agriculture

DUANE K. OKAMOTO
Deputy to the Chairperson

State of Hawaii
DEPARTMENT OF AGRICULTURE
1428 South King Street
Honolulu, Hawaii 96814-2512

April 20, 2010



Robert Pooler
Program Manager
USDA/AMS/TM/NOP
Room 2646-So.
Ag Stop 0268
1400 Independence Ave., SW
Washington, DC 20250

RECEIVED MAY 04 2010

Re: Petition for Formic Acid for Inclusion on the National List of Substances Allowed in Organic Production and Handling.

Dear Mr. Pooler:

The following petition is submitted by the Hawaii Department of Agriculture (HDOA) for the listing of formic acid on the National List of substances allowed in organic honey production and handling.

The petition is prepared and presented in response to specific information that must be provided to USDA, AMS, as noted in Federal Register, Vol. 72, No. 11, Thursday, January 18, 2007, Department of Agriculture, Agricultural Marketing Services 7 CFR Part 205 (Docket No. AMS-TM-06-0223; TM-06-12) National Organic Program – Submission of Petitions of Substances for Inclusion on or Removal From the National List of Substances Allowed and Prohibited in Organic Production and Handling.

# Formic Acid
## Petition for Organic Listing

## §205.603 Synthetic substances allowed for use in organic livestock production

**To be included in a Petition:**

**Item A.** **Indicate which section or sections the petitioned substance will be included on and/or removed from the National List.**

Petitioned Substance:

Formic Acid

Proposed Section for listing:

§205.603 Synthetic substances allowed for use in organic livestock production

**Item B.** **Provide a concise and comprehensive response to the following.**

1. **The substance's chemical or material common name.**

   Material Common Name: Formic Acid

   Chemical Name: Methanoic acid; hydrogen carboxylic acid
   (CAS #: 164-18-6)

2. **The manufacturer's or producer's name, address, and telephone number and other contact information of the manufacturer/producer of the substance listed in the petition.**

   Products Intended for Use:

   Mite-Away II™, EPA Reg. No. 75710-1, EPA Est. No. 075710-CAN-001.

   Registrant:

   NOD Apiary Products Ltd.

2325 Frankford Rd, P.O. Box 117
Frankford, Ontario
Canada, KOK 2CO

Phone No. 613-398-8422
FAX. 613-398-0495

www.miteaway.com

Email: info@miteaway.com

Formic acid is the sole active ingredient.

## Mite-Away Quick Strips™, EPA SLN No. HI-090002

Registrant:

NOD Apiary Products Ltd.
2325 Frankford Rd, P.O. Box 117
Frankford, Ontario
Canada, KOK 2CO

Phone No. 613-398-8422
FAX. 613-398-0495

www.miteaway.com

Email: info@miteaway.com

MAQS has been formulated to work effectively at a higher ambient temperature, thus this product constitutes an unique alternative for tropical areas such as Hawaii

Formic acid is the sole active ingredient.

Food grade formic acid is used to formulate the end-use product.

3. The intended or current use of the substance such as use as a pesticide. If the substance is an agricultural ingredient, provide a list of the types of products (e.g., cereals, salad dressings) for which the substance will be used and a description of the substance's function in the product (e.g., ingredient, flavoring agent, emulsifier, processing aid).

Intended Use:

The intended use is as a pesticide for the control of the varroa mite (*Varroa destructor* Anderson and Trueman (Acari: Varroidae)) in honey bee hives maintained for the purpose of producing certified organic honey.

Formic acid is not currently on the list of approved substances for organic production, i.e., §205.603 Synthetic substances allowed for use in organic livestock production

Formic acid is present in nature in the stings and bites of many insects of the order Hymenoptera, including ants and bees, found naturally in some nectars and some fruits (it helps create acidity which adds to the flavor of coffee beans), and is a natural constituent of honey. The natural product, however, is not available in any sufficient quantity for commercial applications. Synthetic formic acid is chemically identical to natural formic acid and is widely available in laboratories and for industrial applications from suppliers worldwide

Other Current uses of Substance:

Formic acid is used as a preservative and antibacterial agent in livestock feed. When sprayed on fresh hay or other silage, it arrests certain decay processes and causes the feed to retain its nutritive value longer, and so it is widely used to preserve winter feed for cattle. In the poultry industry, it is sometimes added to feed to kill salmonella bacteria. Other uses, include:

- It is used to process latex sap into raw rubber
- Beekeepers use formic acid as a miticide against the Tracheal (Acarapis woodi) mite and the Varroa mite
- It is of minor importance in the textile industry and for the tanning of leather Some formates esters are artificial flavorings or perfumes
- It is the active ingredient in some brands of household limescale remover It is used in laboratories as a solvent modifier for high pressure liquid chromatography separations of proteins and peptides

References:

BASF – http://www2.basf.us/specialtyintermediates/formic_acid.html

FAO Corporate Document Repository: Fish Silage. Torry Advsiory Notes – No. 64, http://www.fao.or/wairdocs/tan/x5937E/x5937e01.htm

Iba, A. M. 1995. Studies on the use of a formic acid-propionic acid mixture (Bio-add™) to control experimental Salmonella infection in broiler chickens. Avian Pathology. 24(2):303-311.

Departemen Perdagangan, Republik Indonesia, ExportNews Indonesia. 2009. Natural Rubber. Vol. 3

Jefferys, D.B. and H.M. Wiseman. 1980. "Formic Acid Poisoning", Postgraduate Medical Journal, 56:761-762.

Waldo, D.R., Keys, Jr., J.E., Gordon, C.H. 1972. Formaldehyde and formic acid as a silage additive. Journal of Dairy Science. 56(2)229-232

4. **List the livestock or handling activities for which the substance will be use. If used for crops or livestock, the substance's rate and method of application must be described. If used for handling, the substance's mode of action must be described.**

**Formic acid in a U.S. EPA Registered pesticide will be used to disinfest honey bee hives of the varroa mite in the production of organic certified honey. Formic acid use in Hawaii will be according to the directions specified solely in the NOD Mite-Away Quick Strips™, EPA SLN No. HI-090002. The directions are as follows:**

**DIRECTIONS FOR USE - Mite-Away Quick Strips™**

It is a violation of Federal law to use this product in a manner inconsistent with its labeling. Do not apply this product in a way that will contact workers or other persons, either directly or through drift. Only protected handlers may be in the area during application. For any requirements specific to your State, consult the Agency in your State responsible for pesticide regulation.

WHEN TO TREAT: Use Mite-Away Quick Strips™ as part of an Integrated Pest Management (IPM) program. Treat only if treatment thresholds are exceeded. Treatment period is for this product is 7 days. Treatment ends at day 7.

When treatment levels are reached, use Mite-Away Quick Strips™ for single or double brood-chamber, standard Langstroth equipment honeybee hives, honeybee colony cluster covering a minimum of 6 brood frames. Outside daytime temperature highs should be between 70 - 92°F on days of application. Excessive temperatures (> 95°F) during the first three days of treatment can cause excessive brood mortality and absconding.

Brood mortality may occur in the initial stage of treatment. Overall colony health is not expected to be affected, with brood rearing returning to normal by the end of treatment. **Treatment of smaller colonies than those listed on the label will result in excessive brood mortality and even in colony mortality.**

APPLICATION Disturb the colony activity as little as possible during the application process. Remove the Mite-Away Quick Strips™ from the outer pouch. For hives with single brood chambers lay two strips across the top bars of the frames of the brood chambers, staggering them so they lay flat and across the full width of the hive body, with approximately 2 inches between strips and 4 inches between the ends of the brood chamber and the outer edges of the strips. For hives with two brood chambers place the strips as described above on the frame top bars of the lower hive body, so the strips are in-between the brood chambers. Put on honey supers, if a honey flow is anticipated. The active ingredient dissipates after 3 days however, do not disturb the colony for 7 days to treatment allow it to recover from any side-effects that may have occurred. Spent strips need not be removed after The bottom hive entrance needs to be fully open for the entire duration of treatment. Other entrances should be

sealed. Entrance reducers MUST be removed to prevent excessive damage to the colonies. Treat all bee colonies in the apiary at the same time. Allow a minimum of one month between applications.

**5. Describe the source of the substance and a detailed description of its manufacturing or processing procedures from the basic component(s) to the final product.**

1. **NOD Apiary Products Ltd. information on the production of Mite-Away quick Strips™, EPA SLN No. Hi-090002 has been shared directly with USDA, NOP to avoid compromise of information considered by NOD to be Confidential Business Information (CBI).**

2. **BASF information on the production of its 95% formic acid has been shared directly with USDA, NOP to avoid compromise of information considered by BASF to be Confidential Business Information (CBI).**

**General Statement Regarding Formic Acid Production**

**Formic acid is a common laboratory and industrial chemical, available from suppliers worldwide. A significant amount of formic acid is produced as a byproduct in the manufacture of other chemicals, especially acetic acid. This production, however, is insufficient to meet the present demand for formic acid, and some formic acid must be produced for its own sake.**

**When methanol and carbon monoxide are combined in the presence of a strong base, the formic acid derivative methyl formate results, according to the chemical equation:**

$$CH_3OH + CO \rightarrow HCOOCH_3$$

**In industry, this reaction is performed in the liquid phase at elevated pressure. Typical reaction conditions are 80°C and 40 atm. The most widely-used base is sodium methoxide. Hyrdolysis of the methyl formate produces formic acid:**

$$HCOOCH_3 + H_2O \rightarrow HCOOH + CH_2OH$$

**Direct hydrolysis of methyl formate requires a large excess of water to proceed efficiently, and some producers perform it by an indirect route by first reacting the methyl formate with ammonia to produce formamide, and then hydrolyzing the formamide with sulfuric acid to produce formic acid:**

$$HCOOCH_3 + NH_3 \rightarrow HCONH_2 + CCH_3OH$$

$$HCONH2 + H2O + 1/2H2SO4 \rightarrow HCOOH + \frac{1}{2}(NH4)2SO4$$

This technique has problems of its own, particularly disposing of the ammonium sulfate byproduct, so some manufacturers have recently developed energy efficient means of separating formic acid from the large excess amount of water used in direct hydrolysis. In one of these processes, the formic acid is removed from the water via liquid-liquid extract with an organic base.

Source of Information: Wikipedia, 7-23-2009

6. **Provide a summary of any available previous reviews by State or private certification programs or other organizations of the petitioned substance. If this information is not available, the petitioner should state so in the petition.**

NOSB Apiculture Task Force Report, September 15, 2001

The NOSB Apiculture Task Force gathered information on materials currently used by apiculture operations and materials currently allowed by certifying agents. The Task Force proposed that ...formic acid ...be reviewed for possible inclusion on the National List, §205.603.

The Task Force report states:

> The Task Force is not endorsing any of the materials listed below (i.e., chart), and is not recommending the approval of any particular material listed. We recommend that the materials listed be reviewed on a high priority basis, due to the fact that many of the materials are currently being used by organic apiculture operations. Without a clear list of allowed apiculture materials, it will be impossible for the apiculture standard to be implemented.
>
> The Task Force recommends that new subsections be created in §205.603 and §205.604 to specifically list synthetic substances for use by organic apiculture operations and non-synthetic substances prohibited for use.
>
> The current materials review process requires that a petition be submitted for each material being requested for review. The Task Force recommends that the NOSB submit the materials listed below for review, and direct the NOP to prioritize their review. The Task Force points out that a similar "blanket" list process was used when crop and handling standards were first developed. The Task Force further points out that this situation will occur when standards are written for any new

sector of the organic industry. Procedures to address the review of materials for new sectors should be developed by the NOSB Materials Committee.

In the table below, the name of the material appears in the first column. The S/N code in the second column stands for synthetic/natural. The third column contains information and notes on how and why the materials are use.

| | | |
|---|---|---|
| Acetic Acid | S | For apicultural use to disinfect empty combs which have been exposed to European foulbrood, Nosema, or the protozoan-caused Amoeba Disease. |
| Carbon Dioxide | S | For apicultural use to control wax moth. |
| Essential oils | N | For apicultural use to control tracheal mites including: menthol, cinnamon, eucalyptus, spearmint, wintergreen, thyme, and camphor. These materials may be used after the last honey harvest of the season and must be discontinued 30 days before the addition of honey supers. |
| Folic acid | S | For apicultural use to control Varroa mites. This material may be used after the last honey harvest of the season and must be discontinued 30 days before the addition of honey supers. |
| Formic acid | S | For apicultural use to control Varroa mites. |
| Lactic acid | N<br>S | For apicultural use to control Varroa mite. This material may be used after the last honey harvest of the season and must be discontinued 30 days before the addition of honey supers. |
| Oxytetracyline | S | For apicultural use. Only for treatment of American foulbrood (AFB) in apiaries in which the disease has been diagnosed; beekeepers may not make routine, prophylactic applications of oxytetracyline in apiaries in which there has been no confirmation of the presence of AFB.<br><br>*(Note: included for discussion purposes because oxytetracyline calcium complex is on the National List for crop production. Although terrmycin is commonly used to control bee diseases, no antibiotics are allowed for other types of organic livestock.) If allowed, an extended withdrawal period or re-transition of the hives should be considered prior to collection of organic apicultural products.* |
| Vegetable shortening | N | For apicultural use to control tracheal mites. This material may be used after the last honey harvest of the season and must be discontinued 30 days before the addition of honey supers.<br><br>*(Note: Some certifiers have allowed vegetable shortening mixed with sugar to form a patty. It is included here for review, but may not need to appear on the list, since it is a natural material and may be used by definition. Since it ends up being eaten by the bees, it is assumed that the shortening would have to be from organic sources. If the shortening is used as an excipient, the Task Force is unclear as to whether the shortening must be organic or if it must appear on the list.)* |

AssureQuality

AssureQuality is a commercial company 100% owned by the New Zealand government. The company provides food safety and biosecurity services to the food and primary production sectors, with independent, impartial, third party auditing, testing, inspection and certification services to organic producers, processors, and retailers in the dairy, meat, seafood, horticulture, wine and arable sectors.

AsureQuality Organic Standard 5.9 Beekeeping and Beekeeping Products, authorizes formic acid use in organic honey production for the control of the Varroa mite, *Varroa jacobsoni*.

Canada

The Canadian General Standards Board, Organic Production Systems Permitted Substance List, allows formic acid for apicultural use to control parasitic mites. This substance may be used after the last honey harvest of the season and shall be discontinued 30 days before the addition of honey supers.

On June 17, 2009, Canada and the United States entered into an arrangement recognizing the national organic systems as equivalent. The equivalency agreement signed by the United State with Canada allows the export of Canadian honey certified organic in Canada to the United State for sale as certified organic. Equivalency is determined by assessing and comparing two regulatory systems, including the standards, to determine whether the principles and outcomes achieved are equivalent. Variations are identified and advice and input are sought from industry on the significance of variations found in the respective standard. Each Government takes this input into consideration while determining if the foreign organic regime can be deemed equivalent. Should either Government identify a critical variance that cannot be resolved, it will become an exception. The June 17, 2009 agreement signed by Canada and the United States includes no specific conditions or exceptions that apply to the use of formic acid for control of parasitic mites in organic honey production. (Source of Information: Canada Food Inspection Agency, Organic Products, Frequently Asked Questions, Canada/US Equivalency Determination or Import/Export Agreement, 7-27-2009)

Europe

The EU-Regulation 2092/91, 1804/1999 Annex C: Beekeeping and Beekeeping Products, states:

Paragraph 6.3 (e)

"without prejudice to the principle in (a) above (i.e., 6.3. The use of veterinary medicinal products in beekeeping which complies with this Regulation shall respect the following principles: (a) they can be used in so far as the corresponding use is authorized in the Member State in accordance with the relevant Community provisions or national provisions in conformity with Community law;) formic acid (emphasis added), lactic acid, acetic acid and oxalic acid and the following substances: menthol, thymol, eucalyptol or camphor can be used in cases of infestation with *Varroa jacobsoni.*

United States

U.S. Environmental Protection Agency Formic Acid (214900) Fact Sheet

"Formic Acid is used to treat tracheal and varroa mites in honey bee hives. There are three registered products. At this time, only one (Mite-Away II) is marketed. The marketed product is formulated as an impregnated pad, and contained in a vented plastic pouch designed to appropriately release formic acid vapors. Although this impregnated pad is safer to use than liquid formulations available in other countries, personal protective equipment is required to preclude potential irritation to eyes, skin and respiratory tract of applicators. It is an alternative to the synthetic pyrethroid and organophosphate products currently in use in the U.S. and is not expected to result in residues above the levels of formic acid naturally occurring in honey (emphasis added). Residues of formic acid are exempt from tolerance requirements. No environmental risk is expected."

7. **Provide information regarding EPA, FDA and State regulatory authority registrations, including registration numbers. If this information does not exist, the petitioner should state so in the petition.**

Two pesticidal products are currently registered by EPA for varroa mite control employing formic acid as active ingredient.

Mite-Away II™, EPA Reg. No. 75710-1, EPA,
NOD Apiary Products Ltd.

Mite-Away Quick Strips™, EPA SLN No. HI-090002,
NOD Apiary Products Ltd.

8. **Provide the Chemical Abstract Service (CAS) number or other product numbers of the substance and labels of products that contains the petitioned substance. If the substance does not have an assigned product number, the petitioner should state so in the petition.**

BASF Corporation
Formic Acid 95% w/w water
CAS #: 64-18-6

9. **Provide the substance's physical properties and chemical mode of action including: (a) chemical interactions with other substances, especially substances used in organic production; (b) toxicity and environmental persistence; (c) environmental impacts from its use and/or manufacture; (d) effects on human health; and (e) effects on soil organisms, crops, or livestock.**

Formic acid is an irritating pungent liquid at room temperature. Ants and bees produce formic acid to help protect them from predators. When used in beehives as directed, formic acid acts by directly killing the mite, while not substantially disrupting bee behavior or life span. The honey bee is less susceptible to the irritating and corrosive properties of formic acid vapor concentrate than the varroa mite. The gel-pack formulation of formic acid is expected to minimize the potential for dermal, eye and inhalation exposure for pesticide applicators. However, because of the corrosive nature of formic acid and potential for eye, skin and mucosal irritation, EPA is requiring stringent precautionary labeling. Exposure and attendant risk are expected to be negligible for applicators when they follow the directions for use, wear the appropriate personal protective equipment and adhere to the restricted entry interval (REI) of 48 hours after treatment and 72 hours in outdoor area where average annual rainfall is less than 25 inches a year.

EPA has examined the potential for formic acid residues to appear in honey and beeswax. When the product is used as directed, residues above those found naturally are not expected. The tolerance exemption for formic acid in honey and beeswax was established in February 1999 (40 CFR 180.1178).

Formic acid is approved for use as a pesticide solely within honeybee hives. Because of this limited use, the Agency does not expect environmental residues to occur outside the hive.

Source of Information: U.S. EPA Formic Acid (214900) Fact Sheet, Issued 4/05

10. **Provide safety information about the substance including a material Safety Data Sheet (MSDS) and a substance report from the National Institute of Environmental Health Studies. If this information does not exist, the petitioner should state in the petition.**

MSDS Attached.

National Institutes of Health, National Toxicology Program Technical Report on Toxicity Studies of Formic Acid in Rats and Mice is attached. No reference could be found for a report from a "National Institute of Environmental Health Studies"

11. **Provide information about the substance which includes comprehensive substance research reviews and research bibliographies, including reviews and bibliographies which present contrasting positions to those presented by the petitioner in supporting the substance's inclusion on or removal from the National List. For petitions to include a non-organic agricultural substances onto the National List, this information item should include research concerning why the substance should be permitted in the production or handling of an organic product, including the availability of organic alternatives.**

Reviews and bibliographies presenting contrasting positions to those presented by the petitioner in support of formic acid for include on the National List:

a. *That formic acid should not be on the National Organic List.*

No references found in support of this position.

b. *That formic acid is not a naturally occurring product in insects and plants.*

No references found in support of this position.

c. *That natural sources of formic acid are available for use for varroa mite control in pesticidal products.*

No references found in support of this position.

d. *That formic acid use as a miticide for varroa mite control in honey production poses an environmental risk, i.e., potential air, soil, and water contamination.*

No references found in support of this position.

e. *That the industrial production (i.e., synthesis) of formic acid can not be undertaken without great risk to the environmental and human health,*

No references found in support of this position.

f. *That the transport of formic acid by air, sea, and by roadway can not be undertaken without great risk to the environment and human health.*

No references found in support of this position.

g. *That registered miticides containing formic acid as active ingredient can not be safely employed by bee keepers for the control of the varroa mite in hives.*

No references found in support of this position.

h. *That registered miticides containing formic acid as active ingredient result in the presence of residues of formic acid in honey that exceed U.S. Food and Drug and/or Codex Alimentarius food safety levels.*

No references found in support of this position.

i. *That formic acid exposure and/or use can result in toxic consequences to man in specific situations.*

Various references found in support of this position.

In methanol ingestion, methanol is oxidized to formaldehyde. The subsequent oxidation of formaldehyde by formaldehyde dehydrogenase results in the formation of formic acid. In acute methanol ingestion, formic acid accumulates and there is a direct correlation between the formic acid concentration and increased morbidity and mortality. The acidosis observed in methanol poisoning appears to be cause directly and indirectly by formic acid production. Formic acid has also been shown to inhibit cytochrome oxidase and is the prime cause of ocular toxicity, though acidosis can increase toxicity further by enabling greater diffusion of formic

acid into cells. Reference: American Academy of Clinical Toxicology practice guidelines on the treatment of methanol poisoning. Barceloux D.G., 2001. J. Toxicol Clin Toxicol. 40(4):415-46.

Formic acid is a strong acid which is available for domestic use as a constituent of proprietary descaling agents and stain-removing fluids. It is therefore a potential source of both accidental and deliberate poisoning. Forty-five cases of formic acid poisoning are described in an article by Jefferys and Wiseman: Jefferys, D.B. and H.M. Wiseman. 1980. "Formic Acid Poisoning", Postgraduate Medical Journal, 56:761-762.

Formic acid decomposes upon contact with strong acids producing carbon monoxide. An article by Chen-Chang Yang, Jiin Ger, and Chun-Fang Li describe a situation where a 26-year old man committed suicide by mixing 2.5 L of formic acid and 2.5 L of sulfuric acid in a closed room. Yang, C.C., Ger, J. and Li, C.F. 2008. "Formic acid: A rare but deadly source of carbon monoxide poisoning, Clinical Toxicology. 46(4):87-289.

Formic acid is applied to hay in silage making. Twelve farmers exposed to formic acid use in silage making were examined for kinetics and renal effects to occupational exposure to formic acid 15 and 30 hours after exposure. Exposure increased renal ammoniagenesis and urinary calcium at 30 – hours post-exposure. The biochemical effects may be explained by the interaction of formic acid with the oxidative metabolism of renal tubular cells, as formic acid is a known inhibitor of cytochrome oxidase. The study concluded that in view of these renal effects, the current hygienic limits may not entirely protect exposed individuals. Liesivuori, J., Laitinen, and Savolainen, H. 1992. "Kinetics and renal effects of formic acid in occupationally exposed farmers". Arch Toxicol. 6:522-524.

Concentrated formic acid slowly decomposes to carbon monoxide and water upon prolong storage, and the gas pressure can be sufficient to rupture sealed glass containers. Chemical & Engineering News Nov. 13, 1989

*j. That the use of formic acid in manufactured products poses a significant health risk of secondary exposure to formic acid.*

No references found in support of this position.

Reference has been found on the safety of formic acid use in manufacturing products, i.e., food handling paper packing products:

"Formic acid is a natural constituent of many foods. It is a metabolite in normal intermediary metabolism, and is a precursor in the biosynthesis of several body constituents. The tolerance of the body to large amounts is relatively high. For example, 160 mg of formic acid per kg per day orally for a period of four weeks; and no adverse effects were reported in rats that received 730 mg of sodium formate per kg in their diet for one and a half years. Average daily intake of ethyl formate and formic acid is about 1 mg per kg or less as formic acid. Although formic acid appears to be moderately mutagenic in *E. coli* and *Drosophila*, ethyl formate is not mutagenic toward strain D4 of *Saccharomyces cerevisiae* or to three strains of *Salmonella typhimurium*. No adverse effects attributable to formate were found in five successive generations of rats given up to 200 mg of calcium formate per kg of body weight daily. Based on these considerations, the Select Committee concludes that: There is no evidence in the available information on formic acid and sodium formate that demonstrates or suggests reasonable grounds to suspect a hazard to the public when they are used as ingredients of paper and paperboard food packaging materials, or as they might reasonable be expected to used for such purposes in the future. There is no evidence in the available information on ethyl formate that demonstrates or suggest reasonable grounds to suspect a hazard to the public when it is used at levels that are now current and in the manner now practices or that might reasonable be expected in the future." The Database of Select Committee on GRAS Substance 9SCOGS) Reviews: Report No. 71, CFR Section 186.1316. SCPGS Opinion, 1976.

12. **Provide a "Petition Justification Statement" which provides justification for any of the following actions requested in the petition:**

**Inclusion of a Synthetic on the National List, §205.603.**

- **Explain why the synthetic substance is necessary for the production or handling of an organic product.**

  Ants are a natural source of formic acid; it is suggested that more formic acid is produced by ants worldwide annually than by industry worldwide annually.

While the above statement may be true, natural formic acid is not available from any known commercial source at a price and quantity that can be used as a miticide in Mite-Away Quick Strips™.

- **Describe any non-synthetic substances, synthetic substances on the National List or alternative cultural methods that could be used in place of the petitioned synthetic substances.**

Organic alternatives:

Powdered Sugar: In the presence of powdered sugar, the varroa mite can be shaken off of the honey bee to be identified and counted. The technique is useful as a survey tool to assess the level of infestation of a hive, but is not feasible as a method for ridding hives of the varroa mite in commercial operations. Rosenbranz, P., Aumeier, P., Ziegelmann, B. 2010. "Biology and control of *Varroa destructor*"J. of Invertebrate Pathology. 103:S96-S119.

Sucrose Octanoate Ester Disinfestation of Hives: Sucrose octanoate esters (CAS #s-42922-74-7; 58064-47-4) – used in accordance with approved labeling is an approved organic treatment for the control of the varroa mite. §205.603 (b)(7).

The mode of action of sucrose octanoate ester treatment appears to be the suffocation or desiccation of the mite. The ester is delivered by spraying adult bees with the substance once every week for three weeks to kill mites as they emerge from brood cells. The method requires significant time, labor and hive manipulation, making it difficult to use in large-scale bee keeping operations. Recent reports suggest the while sucrose octanoate can provide short term increase in mortality of mites, the treatment is not highly effective for the reduction of mite populations in the hive. Rosenbranz, P., Aumeier, P., Ziegelmann, B. 2010. "Biology and control of *Varroa destructor.* J. of Invertebrate Pathology" 103:S96-S119. Barrington.A., Venis, T. 2005. "A new delivery method for Sucrocide, the safe Varroa mite treatment" Am.Bee J. 145(7):583-586. Tarpy,D.R. Summers, J. Managing Varroa Mites in Honey Bee Colonies, North Caroline State University. No 2.04, htttp://www.cals.ncsu.edu/entomology/apiculture/Beekeeping_notes.html

- **Describe the beneficial effects to the environment, human health, or farm ecosystem from use of the synthetic substance that support its use instead of the use of a non-synthetic substance or alternative cultural methods.**

Honey bees are threatened by serious pests, including the varroa and other mites, insects (e.g., small hive beetles) and bacterial and viral disease. The varroa mite is one of the most devastating of these pests that is responsible for the lost of 80 – 95% or more of feral hives in New Zealand and areas of the continental United States. The effective control of this pest and other pests of honey bees is essential to sustain agricultural pollination services and honey production, in the United States. The most effective toxicants for varroa mite control are synthetic pyrethroids and organophosphate compounds. The continued use of these products is threatened by resistance to these chemicals and the tendency for such chemicals to leave residues on wax. The long term effect of these chemical residues, even at sub-lethal doses, on developing honeybees is still not well understood. These chemicals are not natural nor are they available for listing as synthetic products for organic honey production. Formic acid is a commonly used laboratory and commercial substance; at the same time, formic acid is a natural product found most notably in insects as venom and is present naturally as an acid in honey. Formic acid is not available as a natural product for commercial use. Synthetic formic acid is chemically identical to formic acid in insects, including the honey bee. The honey bee is uniquely tolerant of formic acid vapors and the vapor is effective for knock down of the varroa mite on exposed bees (phoretic stage of the mite) as well as the reproductive stages inside sealed brood cells. In the absence of a significant source of natural formic acid, synthetic formic acid is the appropriate alternative and chemically identical product for use in commercial U.S. Environmental Protection Agency and State licensed products for use for varroa mite control in honey bee hives.

Please advise should any additional information be required by USDA, AMS for consideration of this request for the listing of formic acid as an allowed substance in organic honey production and handling.

Sincerely,

Lyle Wong

Lyle Wong, Ph.D.
Plant Industry Division Administrator

P7

01-22-10 A11:56 IN

College of Tropical Agriculture and Human Resourc
**Department of Plant and Environmental Protection Scienc**

UNIVERSITY of HAWAI'I® MĀNOA

To Director: DOA

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Please refer to: 09: 1022215

HAWAII DEPT OF AGR
2009 OCT 27 P 12:07

The Honorable Linda Lingle
Governor, State of Hawai`i
Executive Chambers
State Capitol
Honolulu, Hawai`i 96813

October [illegible], 2009

Dear Governor Lingle,

As you are no doubt aware, the Hawaii beekeeping industry if being severely threatened by the relatively recent invasions of Oahu and the Big Island, by Varroa mites, which are serious parasites of honeybees. Hawaii has an important honeybee industry, with large queen bee producers who supply queen bees to beekeepers in numerous countries. Other important beekeeper contributions to the state agriculture are the provision of pollination services to growers of numerous crops in Hawaii, and the production of organic honey for local and export markets. In June 2008, the University of Hawaii (Department of Plant and Environmental Protection Sciences, CTAHR) received funding from the Hawaii Department of Agriculture to address the new challenges to beekeeping brought on by the arrival of Varroa to the islands.

During the past year and a half the UH Honeybee Varroa project has developed a strong research and extension program that takes advantage of the Varroa management experiences from the mainland but remains focused on the singularities of our local situation. Given that this pest and its impacts on local agriculture receive considerable public attention, we thought it would be valuable to provide an overview of our progress to date, to show what contributions have been made and what challenges we are still faced with.

Enclosed in this package you will find materials our program has produced on beekeeping, honeybees and pesticides, Varroa mite biology and management and the most recent advances in effective treatments for infested beehives in Hawaii. A very significant contribution is the testing of new formic acid treatments for mite control, specific to Hawaii conditions, that has provided very impressive positive results and will contribute substantially to the ability of Hawaii beekeepers to sustain their beekeeping operations even with Varroa mite pressure.

We are continuing to expand our work on Varroa mites, developing locally relevant technology for the management of this problem and looking at the long term environmentally safe management options.

Please feel free to contact us if you require any further information on this issue that is so important to Hawaii agriculture.

Much Aloha,

Dr. Mark G. Wright  Dr. Ethel M. Villalobos

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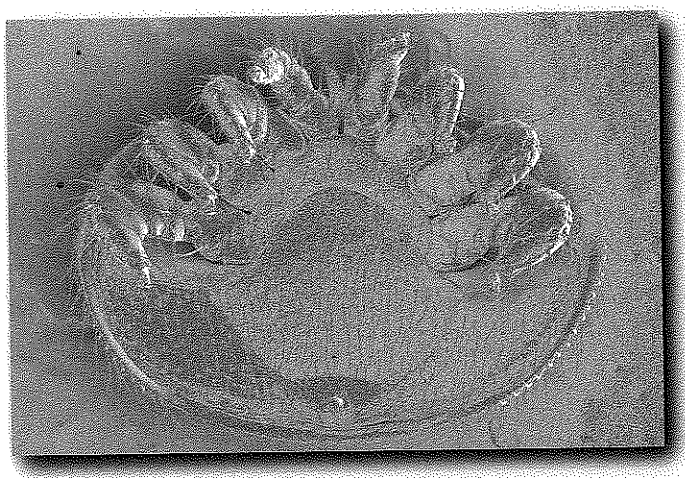
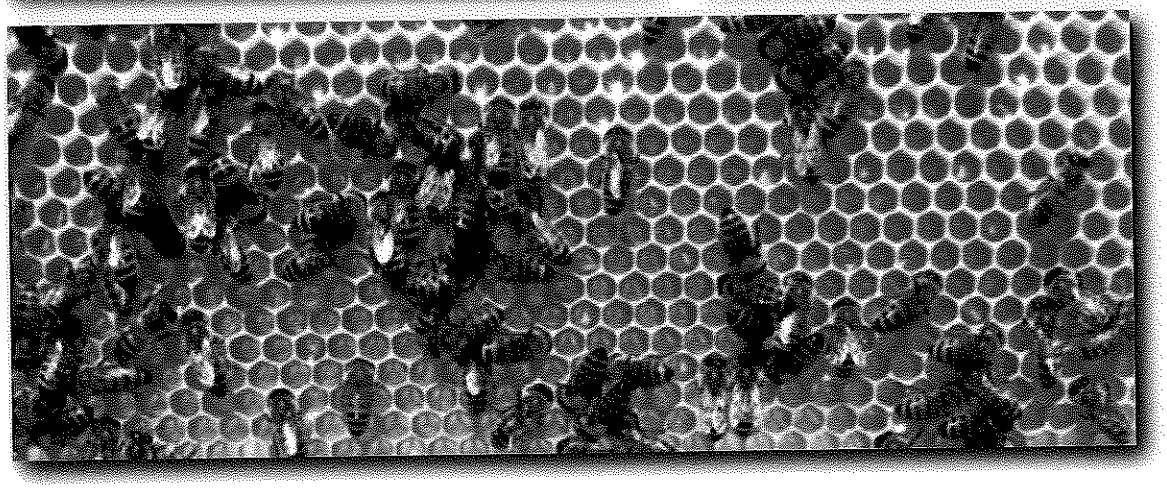
# UH HONEYBEE/ VARROA





## Safeguarding the honeybees

## The impact of varroa on beekeeping in Hawaii

The varroa mite (*Varroa destructor*) is one of the most serious pests of honeybees and it has been associated with the spread of viral diseases and the decline of honeybee colonies in the mainland.

The UH team has been testing the efficacy of a number of organic methods for varroa control. Among the tested management techniques is the selective removal of male bee larva, which are highly attractive to varroa. This biomechanical technique can be a successful management tool for small scale beekeepers, albeit very time consuming. Chemical control using organic substances such as thymol and formic acid are also important elements of an Integrated Pest Management strategy, especially for large scale beekeepers. UH is currently involved in testing a new formic acid product that appears well suited for tropical climates, and may help reduce the impact of varroa on the managed colonies in the islands.

We are also interested in the relationship of varroa and the spread of bee diseases among managed hives and we begun a scientific collaboration with UK researchers to further understand the role pathogens play in honeybee colony decline.

Honeybees pollinate a large portion of our crops. The honeybee-varroa team is involved in extension work with beekeepers and farmers on Oahu and the Big Island of Hawaii. Our goal is to provide information to beekeepers about treatment, encourage newcomers to beekeeping, and provide advice to farmers that have enquiries about the loss of feral bees and the need for pollinators in their fields.

Reducing the likelihood that the mite will invade other islands is also a high priority, and we are collaborating with HDoA in preventing feral bees from being inadvertently transported between islands.


### Contact info.

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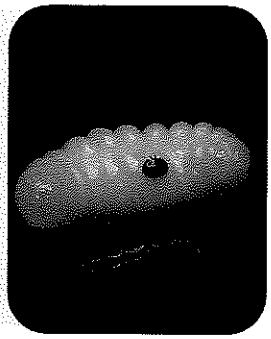
96822

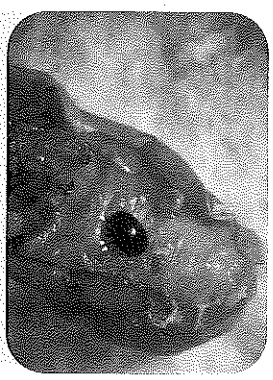
To breed, an adult female enters a cell before it is capped. Once the cell is capped the female will establish a feeding site on the growing bee for herself and her offspring. The females are mated in the cells when they are young, before emerging for the first time.

Each female begins the reproductive cycle by laying a male egg followed by 4-5 female eggs. The male hatches first and mates with the females. If the male dies before mating then the females in the cell will remain unfertilized for the rest of their lives. Males need 5-6 days to develop and females require 6-8.



The varroa mites survive outside the cell by clinging to, and feeding on, adult bees (mostly nurse bees which stay near the brood). They will only re-enter the cell when they are ready to lay their eggs.

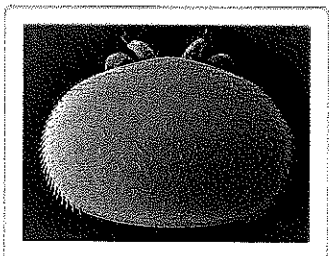
The development of the mite is limited by the length of the developmental period of the bee. This makes drone brood more attractive to the mite than the worker brood since the drones take substantially longer to complete development. When the bee leaves the cell the mature mites do too. Immature mites do not survive. Bee larvae can be attacked by multiple female mites and can cause much damage to the bee.



# Varroa control
# Treatment alternatives and sustainability

The use of synthetic chemicals (fluvalinate and coumaphos) for varroa control is a common practice in many parts of the world. The appeal of the varroacides is their initial high efficacy rate and the ease of application. There are, however, serious drawbacks to using pesticides in hives. Synthetic chemicals are known to leave residues in honey and wax, and beekeepers need to exercise extreme care when exposing their hives to any pesticide. It is also unclear what, if any, effect these chemicals have in the long term health of the developing bees. There is some evidence that miticides may interfere with sperm development in drones and consequently compromise the reproductive ability of the hive. Another concern with the widespread use of miticdes is that the initial efficacy of these chemicals does not remain constant as the mites develop resistance to these treatments over time.

The IPM program attempts to control the mite population using a variety of treatments applied during different times of the year. Successful pest management reduces the losses to honey producers and helps maintain the strength of hives for crop pollination. Although we are not endorsing specific products, we are emphasizing the use of screened bottom boards, drone comb and organic treatments such as thymol and formic acid, which have produced good results during our first year of varroa monitoring and control. We will continue to update our information and the recommendations as more data becomes available for the different regions of Oahu and the Big Island of Hawaii.



# Farming WITH HONEYBEES

Honeybees play an important role in the pollination of many vegetable crops in Hawaii

## Increasing agricultural yields through honeybee pollination

Honeybees live in large colonies and feed exclusively on nectar and pollen. Each colony has many adult bees that search the fields for suitable flowers. In the process of gathering food the bees help spread pollen from one flower to another and in this way they pollinate the crops they visit.

Honeybees help increase yields for many tropical crops. Fruit trees such as lychee, avocados, oranges, and macadamia nuts are examples of bee pollinated plants. Many vegetables, in particular cucurbits such as cucumbers, squash, watermelon, also require bee visits for adequate fruit formation.

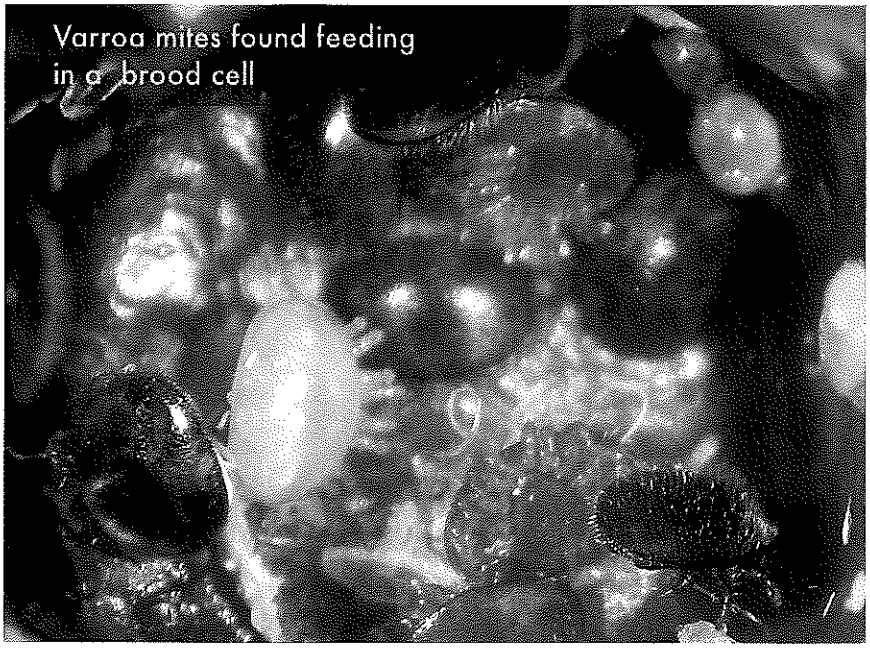
Until a couple years ago Hawaii growers had been fortunate to have many feral colonies that worked their fields for free. However, in 2007, a new pest of the honeybee, the Varroa mite, arrived to the islands and the bees are now scarcer.

A honeybee hive working a hectare of cucumbers can yield 3 times more fruits than plots without bees. Each individual fruit is also heavier in bee pollinated plots compared to those plots without bees.

The number of bee visits a flower receives influences the quality of the fruit that develops. Cucumber flowers must receive 8 to 10 bee visits to produce an acceptable fruit.

UH BEE PROJECT 2009
CTAHR, UNIVERSITY OF HAWAII

If you are interested in having bees in your farm or would like more information about Varroa mites, please visit our website at Bee Project CTAHR
http://www.ctahr.hawaii.edu/wrightm/Honey_Bee_Home.html



Varroa mites found feeding in a brood cell

## Varroa mite biology

The Varroa mite (*Varroa destructor*), a relatively large parasitic mite, ranks among the most destructive bee parasites worldwide.

Varroa mites feed on the haemolymph (blood) of developing larvae and adult bees. Parasitized larvae are weaken or may die of the impacts of being parasitized. Bees may also become infested with deformed wing virus, which is spread by the mites.

The impact of the Varroa mite may be more severe in tropical regions than temperate zones, owing to the fact that honeybees produce brood year-round in the tropics, which permits the mites to continue reproducing year round and consequently the mite populations can increase rapidly in warm climates.

Untreated colonies are weakened by the mite, and may die within a year or two of infestation.

## The impact of the varroa mite on beekeepers and farmers

Feral colonies are significant sources of pollinators, and their decline has negatively impacted agriculture. The sudden disappearance of feral bees has created a need for managed hives at a time when the beekeepers themselves are struggling to minimize their losses.

On Oahu, the lack of feral hives has been felt by many small scale farmers and gardeners. Many growers have even resorted to hand pollinating their crops.

Farmers need to consider if they require bees to maintain a high level of productivity on their farms, and whether they decide to form a partnership with an established beekeeper, or learn how to keep bees for themselves, it is important to select Varroa treatments and insecticides that promote pollinator safe environments and are sustainable in the long run.

Produced by Ethel M. Villalbos, Ph. D. 2009
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University of Hawaii
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## Pesticides and bees

**Insecticides are deadly to bees and it is important for growers to find ways to minimize their destructive impact on pollinators.**

**Here are some tips to reduce honeybee poisoning in your farms:**

1. Avoid spraying crops when they are in bloom

2. Avoid spraying when the bees are most active

   Apply insecticides at "off hours" when the bees are not working your crops to minimize exposure

3. Reduce pesticide drift

   Avoid dust pesticides which tend to drift away from intended locations

   Do not contaminate water resources with pesticides or fertilizers

4. Chemical formulation

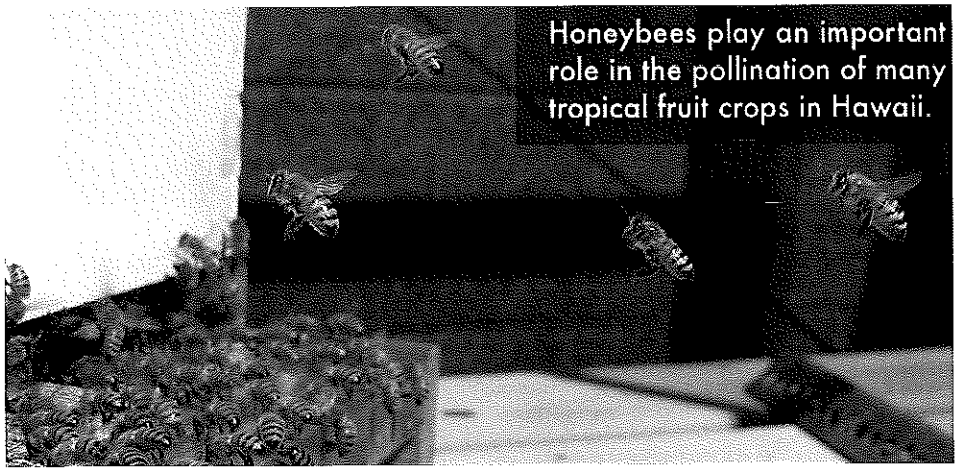
   Use less toxic compounds

Reducing pesticide use is the best way to ensure pollinator safety, however if pesticides must be used, select the least toxic formulations and consider the safest time to apply the pesticide. For more information please visit:

The Xerces Society
http://www.xerces.org/pollinator-conservation/

Pacific Northwest Extension Publication
http://extension.oregonstate.edu/catalog/pdf/pnw/pnw591.pdf

# Farming WITH HONEYBEES



Honeybees play an important role in the pollination of many tropical fruit crops in Hawaii.

## The importance of honeybees as tropical fruit pollinators.

Honeybees live in large colonies and feed exclusively on nectar and pollen. Each colony has many adult bees that search the fields for suitable flowers. In the process of gathering food the bees help spread pollen from one flower to another and in this way they pollinate the crops they visit.

Honeybees help increase yields for many tropical crops. Fruit trees such as lychee, rambutan, avocados, and macadamia nuts are examples of bee pollinated plants. Many vegetables, in particular cucurbits such as cucumbers, squash, watermelon, also require bee visits for adequate fruit formation.

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### Crop yields and bee pollination

Fruit trees vary on their degree of dependency on honeybees for pollination, but even among those crops that can self fertilize, cross pollination by insects often results in higher yields. Lychee, Rambutan, Langan, Starfruit, Avocado, Guava, and Macadamia, have high to medium bee requirements. The number of hives required per acre will depend on many factors including the proportion of male to female flowers in the cultivars and the efficiency of pollen transfer by the bees. A minimum of 1 hive per acre is required, but more often 2 to 3 hives per acre are recommended.

UH HONEYBEE VARROA PROJECT
CTAHR, UNIVERSITY OF HAWAII

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Photos by E. Villalobos and S. Nikaido

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# Varroa Management

*This booklet provides basic information about the monitoring and control of the parasitic mite, Varroa destructor, in Hawaii.*

## The Varroa Mite

*Varroa destructor* is an external parasite of honeybees, that feeds on the blood (haemolymph) of adult and immature bees. The varroa mites originated in Asia, in close association with *A. cerana*, the Asian honeybee. In the 1970's, *V. destructor* was found parasitizing *A. mellifera* in Western Europe. By 1982, the mites had spread to the US. Presently, with the exception of some Hawaiian islands and Australia this destructive honeybee pest is found worldwide.

## Frequently asked questions

### *What are the problems associated with Varroa?*

The varroa mite is one of the most serious pests of honeybees. The mites weaken adult and developing bees by feeding on their haemolymph. In addition high mite levels are often associated with the spread of viral diseases in the hives.

An infested hive is less productive than a healthy hive, and high levels of varroa can kill a colony within a year of infection.

### *When did Varroa get to Hawaii?*

The mites were first detected in Oahu on March 2007, and later found in Hilo, Hawaii, in August of 2008.

### *How do I know if my colonies have Varroa?*

Monitoring for mites can be done in a variety of ways. Some methods are more sensitive than others in detecting the presence of the mite, especially when the mite levels are low.

Sampling adult bees and/or drone brood are the most effective ways to detect the mite. Within this pamphlet you will find a quick review of the most common sampling methods for Varroa.

### *Should I continue to monitor once I know my hives are infested?*

Yes, you should keep track of the infestation levels and treat hives when the mite levels are high.

Mites spend a large portion of their life cycle inside capped cells and their presence might not be obvious at a quick glance. Continuous sampling will help track the levels of non-reproductive Varroa (phoretic mites) that are feeding on the adult bees.

# Monitoring and thresholds

*Survey methods can be used to detect the presence or absence of Varroa or to provide an estimate of the levels of infestation in the hive. High sensitivity in the test is needed for detection. In contrast, less intrusive and rapid methods are often preferred to monitor mite levels.*

## Detection

As the Varroa mite spreads, either via swarms, hive robbing, or more likely through transport of infested hives, the beekeepers in uninfected areas should remain vigilant. Survey methods vary in their sensitivity and their ability to detect the arrival of the mite to an area. There are two sampling methods that increase the chances of detection of the mite: drone brood sampling and adult bee sampling.

Sampling drone brood can be very effective due to the fact that Varroa mites prefer drones 8-10 times more than they prefer worker brood. So even at low infestation levels, drones are a magnet for the reproductive female mites. Sample a minimum of 50 capped drones cells per hive (preferably 100 cells) to increase the odds of detecting Varroa.

If drone numbers are low, sampling nurse bees is a good alternative. When female mites are in their reproductive phase, they are more likely to be found in the cells or on nurse bees, therefore sampling either of these stages increases the likelihood of detection.

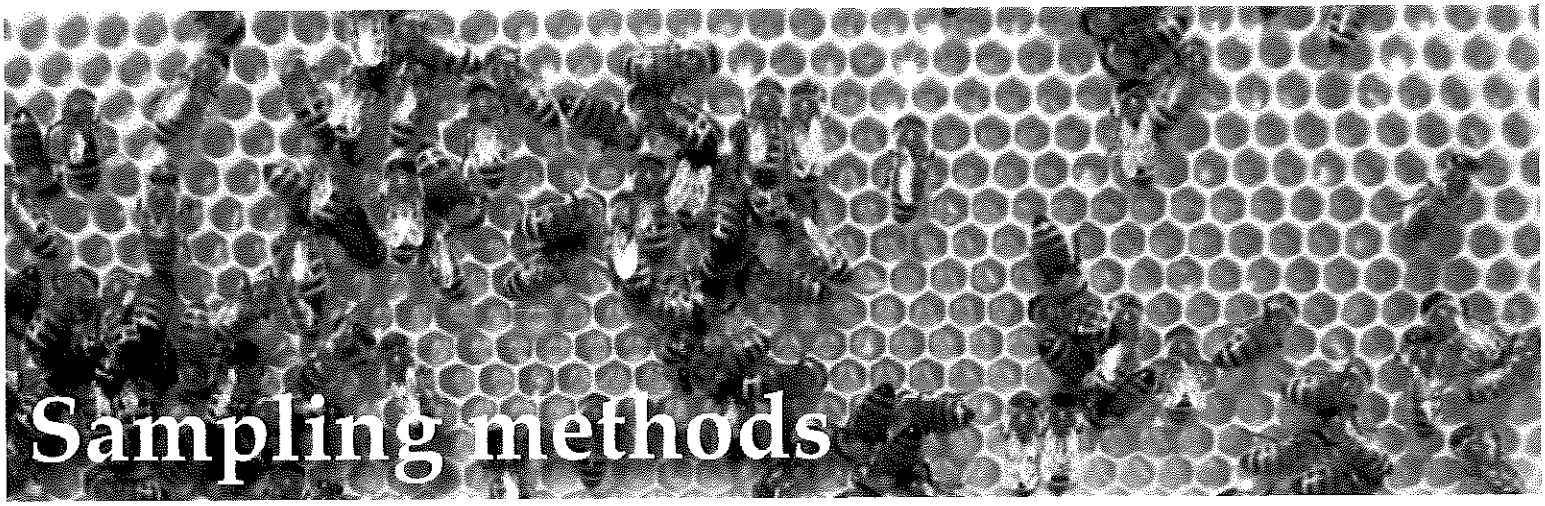
Beekeepers with large bee yards (>100 colonies) are often unable to sample all their hives, and consequently mite detection can be more challenging, The probability of early detection, however, increases if: 1- the sampling occurs at regular intervals (every 1-2 months during the initial stages of Varroa dispersal), 2- the beekeeper makes an effort to sample approximately 10 % of the hives, and 3- the beekeeper includes some of the strongest colonies in the sample.

## Infestation Levels and treatment thresholds

Mite levels have dramatic effects on hive health and survival. A heavily infested hive can suddenly display a number of symptoms, including an increase in viral diseases, such as deformed wing virus, a reduction in brood production, and a quick decline in overall strength possibly due to weakened adult bees. The rapid changes observed at high levels of mite infestation can catch the beekeepers off guard, and a hive can be lost within a few months to a year if the Varroa populations spike out of control.

Although it is obvious that high levels of Varroa can have very serious consequences for the colony and that beekeepers should apply control methods to keep the mite population at a low level, it is somewhat more difficult to determine what can be considered a tolerable level of infestation. Hives vary greatly in the level of infestation that can be tolerated before the colony declines and economic losses occur. Climatic and biological factors such as bee race, and the underlying level of disease that existed in the hive before the arrival of Varroa, may influence the hive's response and tolerance to the mite invasion. It is also possible that the first year or two of Varroa presence may be the more difficult for the hives or that there is year- to- year variation in mite levels.

Beekeepers should monitor and record the cycles of the Varroa population in their own apiaries. The colony cycle table presented below shows an increase of Varroa levels starting in the fall and peaking in winter. During the winter months the mite levels increased 4- fold compared to the summer levels. A noticeable increase in mite levels during the fall suggests that mite populations will spike in the winter and treatment is needed.



# Sampling methods

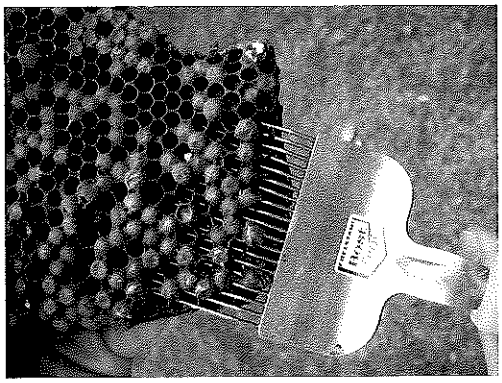
## Sampling adult bees

Sampling adult bees, especially nurse bees will give a snapshot of the level of infestation in your hive. This detection method is based on removing mites attached to adults to produce a count of mites/bee.

To conduct this test, brush about 200 bees from a frame into a clear glass container (mason jar). Cover the jar with a mesh lid. You can then choose to add one of these substances, rubbing alcohol, soapy water, or powdered sugar as a tool to dislodge the mites. Shake or roll (if using sugar) the jar, count the number of mites that fall off. The sugar method is less effective but the bees will not be killed during the sampling.

If you have more than 15 mites/100 bees during the fall, you have a relatively high infestation and should consider treatment immediately.

## Sampling drone brood

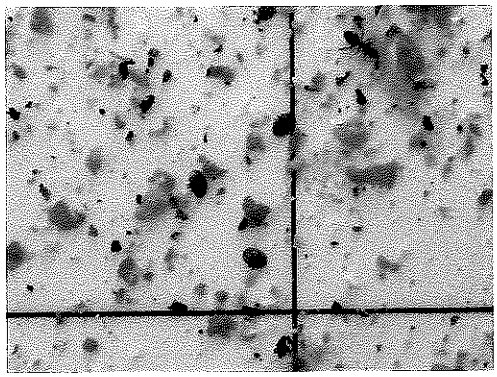


Mite detection can be aided by sampling drone brood. Using a capping -scratcher fork check for mites on the developing drones.

This sampling method is made easier if the drones are clumped in an area of the frame which facilitates the use of the fork or if you can cut away a piece of the brood and examine it for mites. Since female mites are highly attracted to drone brood this method is a very effective early detection tool for Varroa.

Drone brood inspection can also help keep track of the infestation levels in the hive and the beekeeper may also choose to use drone brood removal as a Varroa management tool (see below for details).

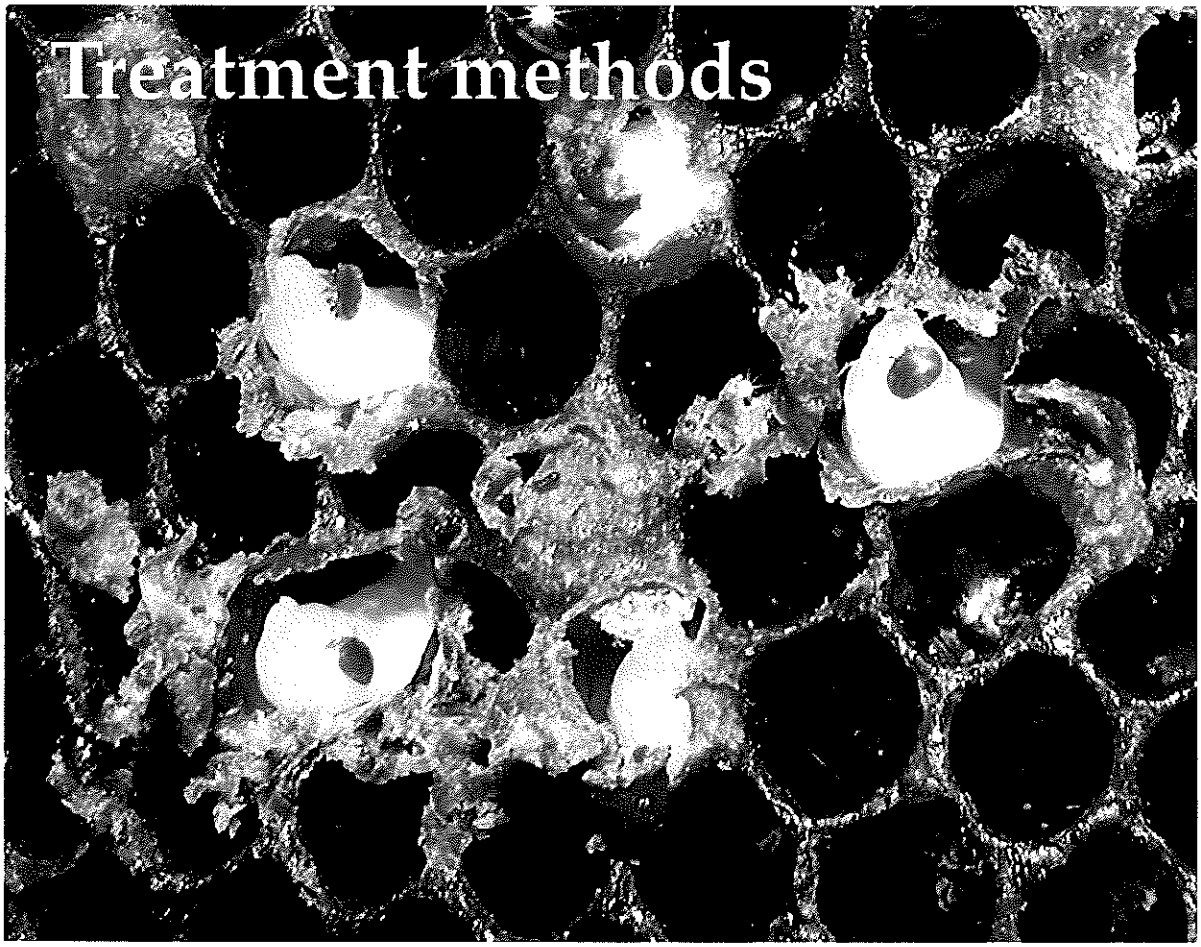
## Using sticky boards



Using screened bottom boards in combination with some kind of sticky surface to trap fallen mites is a recommended technique for management and sampling. Recording the number of mites that fall off the comb onto a sticky board (passive mite drop) is a relatively easy technique that provides a record of mite levels. This method is simple and non-disruptive. Unfortunately it is not very sensitive to low levels and may not be the best choice as an early detection method.

***If you would like more detailed information about how to conduct these sampling techniques, please go to our website and download the handout on Varroa sampling and detection.***

# Treatment methods



## WHAT METHODS ARE AVAILABLE TO CONTROL VARROA?

*Control methods can be divided into:*

- ***Chemical***

  *Synthetic: varroacides.*

  *Natural: organic oils and acids.*

- ***Biomechanical***

  *Chemical free techniques that can be used during the honeyflow. Examples are drone comb removal and screen bottom boards.*

- ***Cultural***

  *The use of management techniques to disrupt the reproductive cycle of the colony and the mite.*

- ***Genetics***

  *Selection and breeding of traits than reduce Varroa's impact on the colony.*

*Please note that beekeepers may choose to use a mixture of controls methods as part of an Integrated Pest Management strategy, please see below for more details about this practice.*

## HOW DOES THE CLIMATE OF HAWAII INFLUENCE THE TREATMENT METHODS I CAN USE?

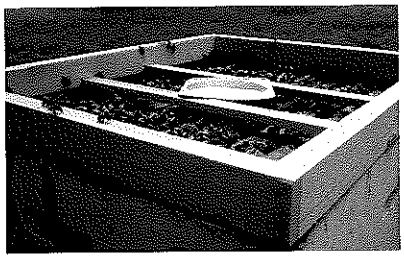
*The Hawaiian climate allows the bees to produce brood year round, consequently treatments designed to work during the broodless periods, typical of temperate regions, are not suitable for the local conditions. Products such as lactic and oxalic acid are of little use for Hawaii beekeepers.*

*The prolonged flowering season of Hawaii results in extended honey flows and multiple honey harvests throughout the year. Varroacides however, should not be employed when there is a honeyflow and local beekeepers need to recognize when a suitable "window of opportunity" is present for treatment to avoid honey contamination.*

*Organic treatments such as thymol and formic acid can be employed in warmer climates, but the beekeepers should avoid excessively warm humid periods. Selecting the right environmental conditions is crucial for the efficacy of any control method.*

# Thymol gel (Apiguard)

*Thymol is one of many essential oils that show promise in controlling Varroa infestationsyThymol is sold under the trade name Apiguard and is applied as a gel formulation delivered in a tray to the top of the brood chamber. The product works by both contact and evaporation (sublimation) of the chemical. The honey supers should be removed during application, and for best results we recommend that treatment be applied when ambient temperatures are 59 F⁰ to 85 F⁰. The hive cover needs to provide enough air space for the chemical to sublimate and disperse homogeneously throughout the colony. We suggest a 2 inch space between the top of the brood frames and the cover.*



*Reports for thymol efficacy are generally high, ranging from 70 to 90% mite kill. Our experience with thymol indicates that best results are obtained if the treatment tray is introduced in the late afternoon, and the initial sublimation takes place in the evening when ambient temperatures are lower. We also noted, contrary to some recommendations, that the hive's entrance should not be closed and screen bottom boards can be used during treatment. Avoid rainy periods since adult bees may initially congregate on the outside of the hive when thymol is introduced.*

*Beekeepers should keep an eye on treated hives, because thymol applications may temporarily disrupt the egg laying of the queen, and rarely the queen may disappear or die. If applied correctly the treatment is most often successful, and mite drops are very high.*

*Hives with 30,000 bees or more should be able to handle the full thymol dose of 50g per tray. Treating smaller hives or nucs may require adjusting the dose of the chemical. Hives with heavy infestations may require 2 consecutive treatments with thymol.*

# Drone comb removal

*The drone comb treatment aims at trapping mites as they attack the brood. Drone cells are known to be significantly more attractive to female mites compared to worker brood cells. Selectively culling the capped drone brood provides both a way to estimate overall density of infestation but also serves as an effective control method by removing a large proportion of the mites found in the colony.*

*Plastic drone combs or drone foundation can be purchased commercially or beekeepers can modify a regular frame into a "half frame" with foundation only on the top part of the frame. Half frames allow the bees to draw drone cells in the open bottom section of the frame, and the drone portion can be simply cut away with a knife and removed.*

*The key to the success of drone comb trapping depends on: 1- the amount of drones produced by the hive and 2- the beekeeper's timing of the removal of the frame. Drone frames need to be removed after the majority of cells are capped, in order to trap mites within the cell, but before drone emergence, in order to prevent re-infestation by the mites and her newly formed daughters. If the drone comb is not removed promptly the hive will become a virtual "Varroa nursery". Deciding when to remove a drone frame takes practice, beekeepers can aim for 25-30 days after the frame is introduced to the hive. On Oahu, Varroa infestation levels in drone brood average 30 to 40 % in the summer months and removal of full drone frames (approximately 1400 drones) can have a large impact on the mite population.*

# Using varroacides and the development of resistance

*The use of synthetic chemicals (fluvalinate and coumaphos) for Varroa control is a common practice in many parts of the world. The appeal of the varroacides is their initial high efficacy rate and the ease of application. There are, however, serious drawbacks to using pesticides in hives. Synthetic chemicals are known to leave residues in honey and wax, and beekeepers need to exercise extreme care when exposing their hives to any pesticide. It is also unclear what, if any, effect these chemicals have in the long term health of the developing bees. There is some evidence that miticides may interfere with sperm development in drones and consequently compromise the reproductive ability of the hive. Another concern with the widespread use of miticdes is that the initial efficacy of these chemicals does not remain constant as the mites develop resistance to these treatments over time.*

*Beekeepers that use varroacides should follow label directions carefully and avoid applications during the honey flow. To delay the development of resistance the beekeepers should treat only for the specified time and only when the mite levels require treatment. In addition, beekeepers should consider alternating their varroacide treatment with other chemicals, such as thymol or formic acid, or even including biomechanical control methods like drone brood removal in their management schedule.*

## The relationship between colony cycle and Varroa management

The life cycle of the Varroa mite is linked closely to the life cycle of the bees. The large- bodied, slow-developing drone bees provide excellent hosts for the female mites. Drone brood allows individual female mites to produce more offspring, and each drone can support more than one reproductive female mite.

Just as the life cycle of an individual mite matches that of its host, the growth of the mite population is linked to colony growth cycles. The availability of brood, and drone brood in particular, has a great impact on mite abundance. Although brood is always available in Hawaii's tropical climate, the reproductive cycle of the colony and certain management techniques can affect the availability of brood and consequently the population growth of Varroa. For example, during the swarming season, as the drone numbers increase, mite numbers also begin to rise. On Oahu, there can as much as a 10-fold increase in drone production during the summer months. In contrast, during re-queening events, when there is a decrease in egg laying and a temporary reduction in brood availability (worker and drone alike), mite numbers fall temporarily. Consequently, adjusting the treatment schedule to the cycles in brood production and anticipating variations in mite levels are important components of Varroa management.

Another important element affecting Varroa population growth is mite reproductive success, which is determined by the mother mite's ability to produce fertile daughters. Research has shown that mites from different parts of the world differ in their fertility, with some strains having lower reproductive success than others. We need to gain a deeper understanding of the dynamics of the mite population here in Hawaii so that we can better predict their increase and adjust our treatment levels. Research on this important issue is ongoing at UH Manoa.

## Integrated Pest Management of Varroa in Hawaii

Integrated Pest Management (IPM) is a strategy commonly used in agriculture to ensure crop productivity while keeping pesticide use at a minimum. The goal of an IPM program is to intervene before the pest population increases and causes economic damages to the producer. Although IPM techniques aim to reduce the pest density, the treatments are not expected to eliminate the pest completely.

In the present situation with Varroa, the IPM program will attempt to control the mite population using a variety of treatments applied during different times of the year. Successful pest management will reduce the losses to honey producers and will help maintain the strength of hives for crop pollination. Varroa IPM strategies are likely to vary from locality to locality due to weather, colony cycles, and honeyflow seasonality. It is important that each beekeeper becomes familiar with the rhythms of their hives and is able to identify the reproductive cycle and the corresponding rise in the mite population. The charts presented in this brochure are based on data from the southeastern portion of Oahu.

Although we are not endorsing specific products, we are emphasizing the use of screened bottom boards, drone comb and thymol, treatments that have produced good results during our first year of Varroa monitoring and control. We will continue to update our information and the recommendations as more data becomes available for the different regions of Oahu and the Big Island of Hawaii.

# Colony dynamics

*Colon□d□namics will vary slightly across the islands and each beekeeper should consider his/her unique situation. The key issue is to recognize when mite levels are increasing and treat accordingly. To able to identify and respond to changes in mite density the beekeeper needs to sample at least 4 times during the yea*

| Colony Dynamics | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Worker Brood | | | | | | | | | | | | |
| Drone Brood | | | | | | | | | | | | |
| Honey Flow | | | | | | | | | | | | |
| Swarming Season | | | | | | | | | | | | |
| Re-queen/Spliting | | | | | | | | | | | | |
| Mite Levels Passive Drop | | | | | | | | | | | | |

High

Medium

Low

# Treatment and sampling schedule

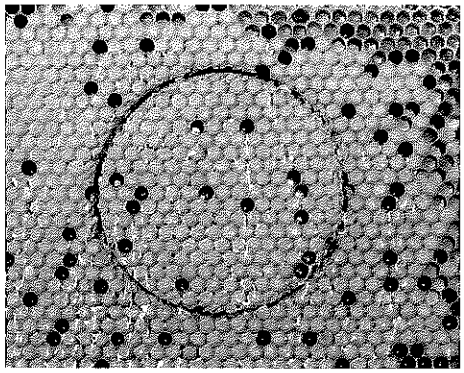
| Control Method | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Screan-bottom-board | | | | | | | | | | | | |
| Drone Brood Removal | | | | | | | | | | | | |
| Thymol Treatment | | | | | | | | | | | | |
| Formic Acid Treatment | | | | | | | | | | | | |
| Varroa Mite Sampling | | | | | | | | | | | | |

Treatment

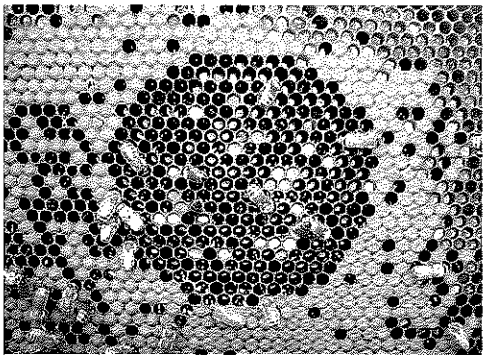
No Treatment

## Hygienic Behavior: How genetics can play a part in the control of Varroa

Traits that are important in the breeding and selection of managed honeybee colonies include productivity, tameness, and behavioral mechanisms that increase colony resistance to disease. Hygienic behavior is the genetically based tendency for the worker bees to detect and remove larvae that are infected with diseases and parasites, including Varroa. Hives are considered hygienic if the workers remove 95% of dead or infected pupa within 24-48 hours. To test for hygienic behavior a patch of capped brood of about 200 cells is killed by freezing. The bees' response to the dead larvae and pupae is then recorded and scored.



Patch of brood frozen with liquid nitrogen (above), and the same patch 48 hours after re-introduction to the hive (below).



According to the literature, the frequency of hygienic behavior in the wild is relatively low, approximately only 10-12 % of hives exhibit this trait. On Oahu, the percent of hives that exhibit hygienic behavior is very variable, but some hives appear to be consistently good throughout the year. Identifying hives with strong cleaning behavior, especially among those that have already been exposed to Varroa, is highly recommended. These hives can be used as a source of new queens and drones and to produce splits. In addition to the natural resistance found in some hives, beekeepers may want to consider locally produced queens fertilized with semen from hygienic drones. These queens should produce consistently good cleaning worker bees and thus provide some added protection against the impact of the Varroa mite.


**Plant and Environmental Protection Sciences**

**College of Tropical Agriculture and Human Resources**

University of Hawai'i at Mānoa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http://www.ctahr.hawaii.edu/wrightm/Honey_Bee_Home.html

Or, Google search for "bee CTAHR".


Written by Ethel M. Villalobos. July 2009. Thanks to M. G. Wright, T. Shelly, L. Medina, S. Nikaido, and J. Wright for comments and suggestions in the making of this brochure. Photos by E. Villalobos, S. Nikaido, and J. Wright. Funding provided by the Hawaii Department of Agriculture.

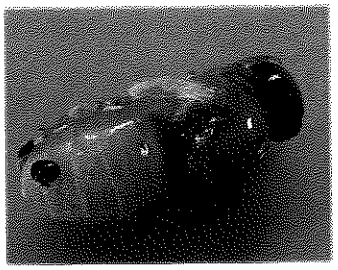
# VARROA MITE

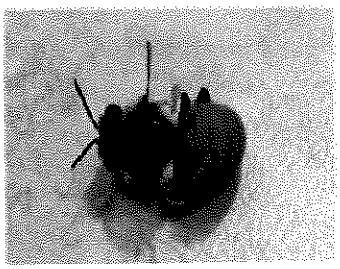
## DETECTION AND SAMPLING



The varroa mite (*Varroa destructor*) is a serious pest of honeybees. As an external parasite, the varroa mite feeds on the hemolymph of adult workers, drones, and developing honeybees. Reproduction of varroa occurs within the capped cells of developing honeybees, with drones having a higher infestation rate than worker bees. Colonies infested with varroa will show symptoms of varroasis: spotty brood, disfigured and deformed wing bees. Within 2 years, a colony with varroa can collapse and die. Varroa was first detected in the United States in Florida in 1987 and quickly spread to other states throughout the country. Due to geographical isolation, Hawaii was free of this parasite for many years. However, in 2007, the mites were detected in Hawaii on the island of Oahu, and soon after, on the island of Hawaii in 2008.

It is important for beekeepers to detect the arrival of varroa to their apiaries and to subsequently monitor their yards regularly for changes in varroa mite levels. Sampling for mites is an important part of any IPM strategy to combat varroa, as infestation levels will help determine if and when colonies are treated. Keeping varroa mite levels low will promote colony health and productivity.

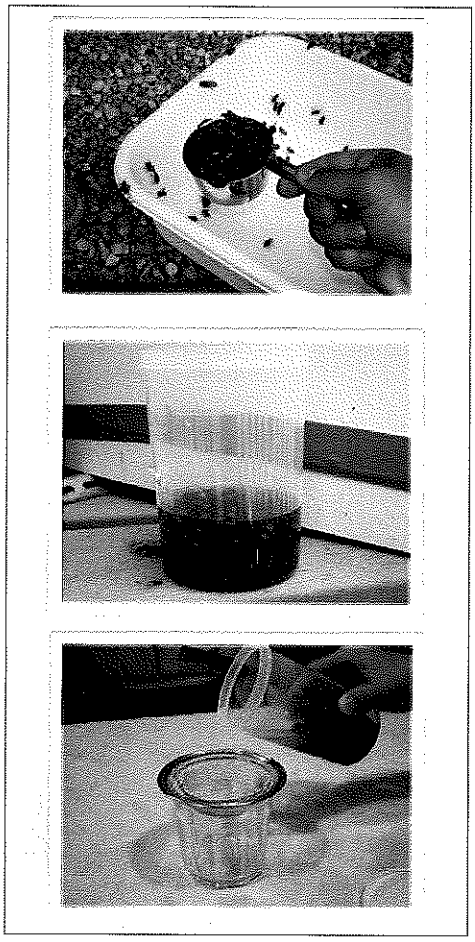




### Varroa and Viral Diseases in Honeybees

*Varroa mites are vectors of viral diseases that affect the development of honeybees. At high infestation levels, symptoms (spotty brood pattern, shrunken abdomens, and deformed wings of worker and drone brood) from viruses can be seen throughout the colony.*

# METHODS



## Alcohol / Soap Shake

The alcohol or soap shake targets phoretic mites that are attached to adult honeybees. Mites are dislodged and killed, then separated from the honeybee sample. This method sacrifices approximately two hundred bees from the colony.

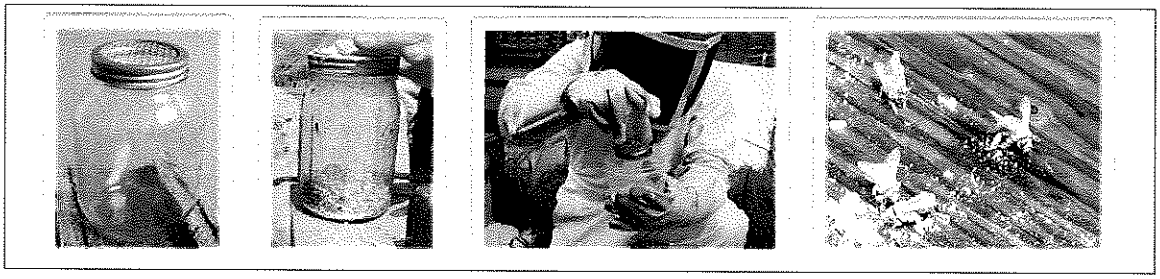
This sampling method is quick and accurate, especially when brood levels are low and the majority of the mites can be found feeding on the adult bees.

- Brush or shake approximately two hundred bees into a glass or plastic jar containing two hundred milliliters of alcohol (isopropyl or ethyl) or dishwashing detergent soap.
- For a precise measurement, shake bees into a bucket, then scoop up the bees with a measuring cup (1/2 cup = 200 bees).
- Shake jar vigorously for one minute.
- Filter contents through a sifter or a wire mesh screen (#8, 3 mm) and examine for mites.
- Additional rinsing with water may be required as mites may still be attached to the wings and/ or other body parts of the honeybee.

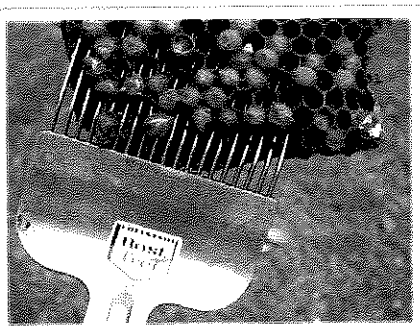
## Powdered Sugar Shake

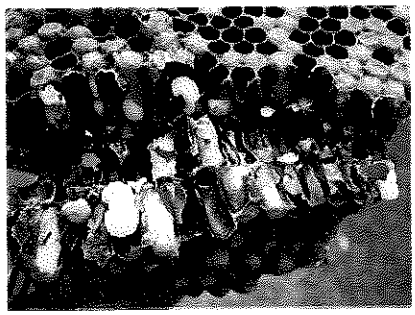
Powdered sugar can be used to dislodge mites from adult bees. Unlike using alcohol or soap, powdered sugar is a non-lethal method and will not kill the bees in the sample. Coating the bees in powdered sugar will stimulate grooming behavior in bees, thus removing mites. In addition, powdered sugar clogs the tarsal pads causing the mites to lose their grip and dislodge from the adult bees. Not all of the mites in the sample will necessarily be removed from the bees. Approximately 10% or more of the mites may remain on the bees.

Modify the cover of a wide mouth Mason jar by installing a #8, 3 mm wire mesh screen. Brush or shake approximately 200 bees into the Mason jar. Pour 1 teaspoon of powdered sugar through the cover of the wire mesh. Roll the jar ensuring each bee is coated in powdered sugar. Invert it and shake vigorously into a container (white is preferable). Count the number of mites that fall. Open the jar, and pour out bees in front of the hive entrance.



# METHODS





## Brood Sampling

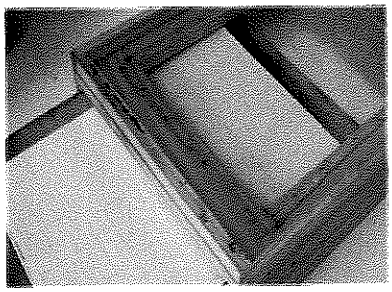
During brood production, approximately 80% of varroa reside in the cells of brood larvae. Taking a random sample of brood larvae can be used to detect the presence of mites in a colony. Since varroa mites prefer drone cells over worker cells, sampling drone brood can give an accurate estimate of the infestation level. However, at low level infestations, detection of the mite can be difficult. Drone sampling is obviously limited by the reproductive cycle of the colony.

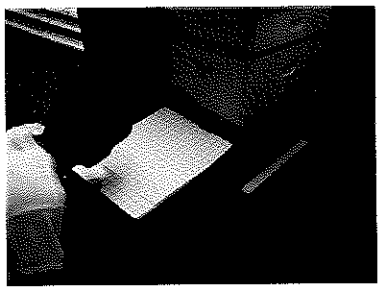
- Use a capping scratcher to remove 50 – 100 drone pupae. If no drone cells are available, worker pupae can be sampled.
- Search for mites on each individual pupae or cell. Feces (white spots) can also be present in cells containing mites.
- Since mites prefer to utilize drone cells, a drone frame (an empty frame can also be used) can facilitate the detection of varroa mites.
- Once the frame is used and the drone larvae are capped, remove the frame and sample larvae with a capping scratcher.

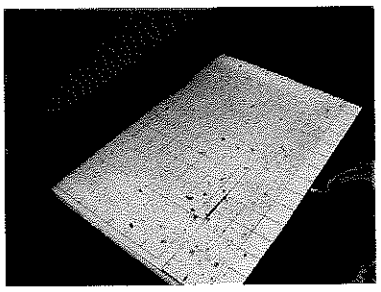
## Mite Fall

Varroa mites will fall off of honeybees due to death, or by honeybee grooming. This passive mite fall can be used as a detection method as well as a means of monitoring the varroa levels in a colony. A sticky board is placed below a screen mesh allowing for daily or weekly counting of varroa mite. To increase mite fall for detection of a new or low infestation, powdered sugar, or an acaricide can be used in conjunction with the screen bottom.

Install a screen bottom board (#8, 3 mm wire mesh) with a sticky board below the screen. A poster or plastic board can be covered with any sticky substance (Tanglefoot, Crisco, or Vaseline) and be used to trap mites after they fall through the screen. After one to seven days have passed, remove the board and examine it for mites. Leaving the board under the screen for over a week can result in lots of hive debris accumulating on it and can make detection difficult.



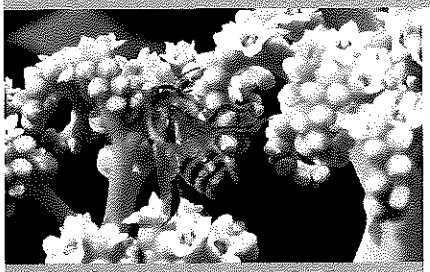




Produced by Scott Nikaido and Ethel M. Villalobos
Plant and Environmental Protection Sciences, CTAHR, University of Hawaii
Photos by S. Nikaido, E. Villalobos, and E. Shelly
Design by Jonathan Wright

# Report on the efficacy of Mite Away™ Quick Strip

An experimental test conducted by the University of Hawaii



## KEY POINTS

### FORMIC ACID

Formic acid is an organic acid (compound) that is found naturally in some animals, such as ants, and it can also be found at low levels in honey. Formic acid can be used as a bio-pesticide for *Varroa destructor* control.

### SUMMARY OF RESULTS

The new NOD product produced a large initial "downfall" of phoretic mites from the hives.

Mite mortality under capped cells was also very high.

Although adult bees initially moved towards the hive entrance, this effect was temporary and no absconding was observed during the treatment.

There was minimal brood damage in treated colonies.

Bees under capped brood were unharmed by the formic acid application.

There was no queen loss during the month long trial.

Egg laying resumed immediately after the treatment.

The new NOD MAQS performed very well under the Hawaiian summer conditions.

The availability of a warm weather formic acid alternative is a much needed tool for *Varroa destructor* control in Hawaii.

## Hawaii field tests of formic acid for Varroa control

Under an experimental permit from the Hawaii Department of Agriculture the UH Honeybee Varroa Project tested Mite Away™ Quick Strip (MAQS), a new formic acid treatment created by NOD Apiary Products Ltd. (Canada) in three apiaries located on the island of Oahu.

Formic acid has been used as an organic miticide to control varroa and appears to have a high degree of selectivity, that is, formic acid is more likely to kill mites rather than harm the bees when applied in the correct dosage and under suitable temperatures. High ambient temperatures are often associated with an increase in bee deaths and brood damage during formic acid applications. Tropical areas, such as Hawaii, frequently experience temperatures much higher than recommended for formic acid products and consequently have fewer options for summer treatments. The new NOD formulation is designed to work at the hive's core temperature and, consequently, is affected less by high ambient temperatures and/or extreme temperature fluctuations found outside of the hive.

## Application and mode of action

We tested a single application of MAQS to the brood chamber. MAQS does not require hive spacers of any sort, and can be applied directly over the brood frames.

The beekeeper must wear a respirator and use acid resistant gloves during application. The screen bottom boards must be covered during the treatment.

Test hives remained open through the treatment, even entrance reducers were removed, to allow for enhanced air circulation and gas dispersion within the hive.

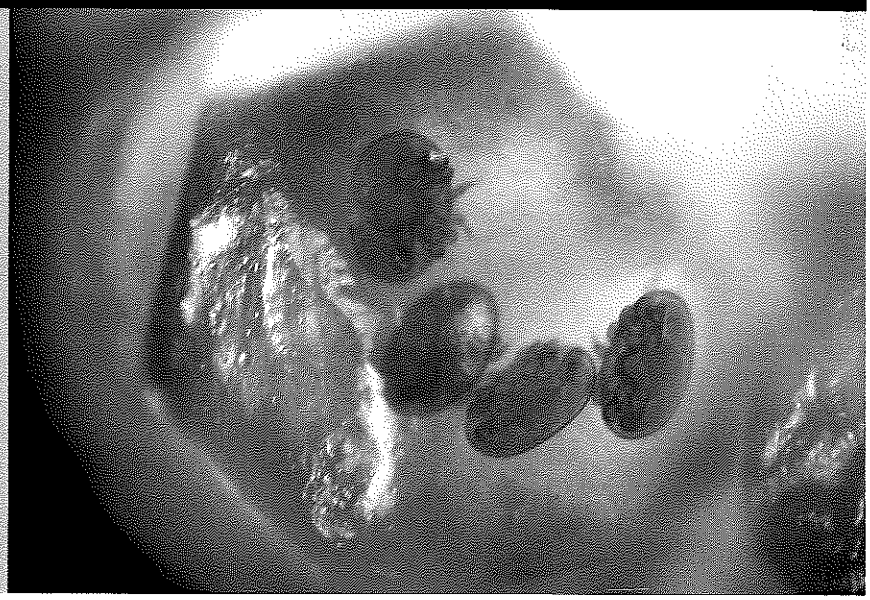
How it works: Formic acid vapor is released from the MAQS matrix saturating the hive space. By the end of the three day application period all the formic acid has been released and all that remains is an inert matrix.

Formic acid and thymol, two commonly used fumigants, vary in their efficacy depending on the ambient temperature during the application. The product that we tested is expected to have a more consistent rate of release. The rate at which vapor is released is influenced more by the constant temperature of the hive than by ambient temperature.

# THE FIELD TEST

## TREATMENT GROUPS

We included in the trial a total of forty two hives of various colony strengths, the hives belonged to UH and to three local beekeeper collaborators. The experimental hives were assigned to either a control group or one of three treatment groups. The treatment groups consisted of a low dose treatment with no supers, a high dose treatment also without supers, and finally, a high dose treatment with the honey supers on.



## Treatment groups and assessment of the hives

The trial included a total of forty one hives of various colony strengths. The experimental colonies were assigned to either a control group or one of 3 treatment groups. The treatment groups consisted of a low dose treatment with no supers (n=6), a high dose treatment also without supers (n=7), a high dose treatment with the honey supers on (n=15), and a control group (n=13).

To evaluate the impact of each treatment on the colonies, we inspected, prior to the start of the trial, each frame and noted the amount of brood present. We also recorded the mite infestation levels via passive mite drop on sticky boards for 3 days before the start of the trial.

The efficacy of formic acid treatments can be influenced by ambient temperature, relative humidity, the hive configuration (hive space), and the positioning of the product within the hive. Ambient temperature, core hive temperature, and relatively humidity were monitored during the trial application using HOBO units (a device used to monitor and log temperature and/ or humidity). Recording units were installed inside the brood box of one control hive and two test hives, and the ambient temperature and relative humidity at the apiary were monitored using a fourth HOBO unit placed in the shade of a macadamia tree. Two dosages of formic acid were tested in the trial, 200g and 300g strips. The weight of the formic acid strips was recorded in the field prior to insertion in the hive and immediately after removal from the hive.

Evaluation of treatment efficacy included both data on mite mortality and observations on the bees' response to the treatment. Mite mortality after formic acid treatment was evaluated by comparing the mites that fell on sticky boards (control versus treatment) and by dissection of capped cells to observe the survival of adult and immature mites inside brood cells. Mite mortality was assessed throughout the month-long experiment by monitoring mite drop levels every three days initially, and weekly after the first seven days of the trial. The three day treatment period when the formic acid was inside the hive will be referred to as "at treatment" hereafter, and data from sticky boards collected at the end of this period constitute our first measurement of product efficacy. Colony response was evaluated by noting the degree of brood damage immediately after treatment, queen presence or absence, and egg laying activity post treatment. The long term effects of the treatment will be evaluated by recording before and after treatment the levels of formic acid in honey and the overall hive strength.

***Please note:*** The bees respond to formic acid by initially exiting the hive and fanning. This mass exit can be alarming to observe, but during the experiment the bees re-entered the hive within twenty-four hours.

# RESULTS
## Control efficacy

### Mite levels and product efficacy

All experimental colonies had similar levels of varroa infestation at the beginning of the trial, as indicated by the pre-treatment sticky board counts ($p>0.05$, Kruskal-Wallis test). The average colony had a passive mite drop of 49.4 mites/day, which is typical for this season on Oahu.

The mite drop at treatment varied significantly among the four categories of hives, Fig. 1 ($P= 0.003$. $H=14.3$, $DF = 3$ Kruskal-Wallis test). Subsequent analysis using Dunn's test revealed a significant difference between the High Dose with Super category and the control ($P < 0.05$) but no significant differences between the other 2 formic acid groups and the control or among the three formic acid groups ($P > 0.05$ in all cases). Treated colonies however, had a combined average mite drop of 223 mites/day compared to 51 mites/day in control colonies. This initial mite drop observed at treatment most likely represents the phoretic mite kill obtained with these formic acid applications.

Formic acid is one of the few varroa control methods that can cause the death of the mites while still under capped cells. It is the killing ability of formic acid that makes it possible for the treatment to be of short duration (three day application), thus reducing the treatment's possible interference with honey collection.

Three days after the formic acid was removed from the hives, mites that had died within the capped cells during treatment would be exposed as the new bees emerged from the cells. The dead mites would then be removed by the nurse bees and contribute to the counts on the sticky boards. When sampled after three days, a treatment successful at killing mites under capped brood cells should yield a higher number of mites on the sticky boards of the treated colonies than for control colonies.

The cumulative mite drop of test colonies, from the start of the trial to three days after treatment, varied significantly ($P=0.011$. $H=11.2$ $DF=3$, Kruskal-Wallis test). The cumulative mite fall for the "High Dose with Supers" category was significantly higher than that of control colonies ($P<0.05$, Dunn's test) Fig. 2. The treatment groups, however, did not differ significantly in their cumulative mite drops at this point of the trial ($P>0.05$ Dunn's test).

**Figure 1: Average mite drop per day**

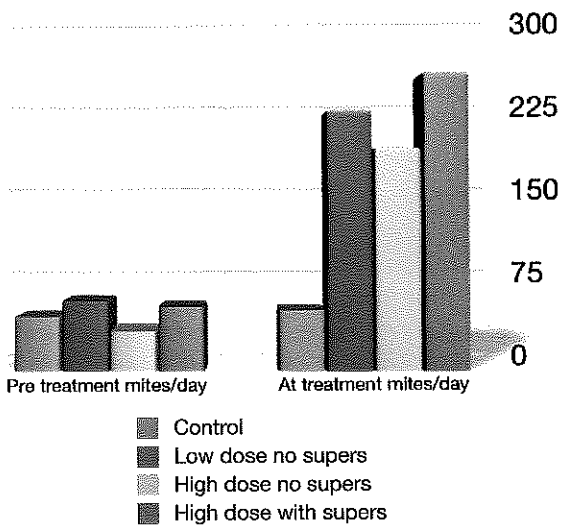
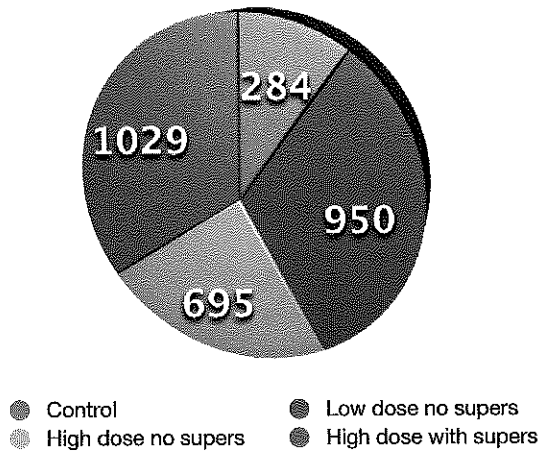



**Figure 2: Cumulative mite counts at three days post treatment**



# RESULTS
## Control efficacy

### Mite levels and product efficacy

The application of the NOD MAQS resulted in a large initial kill of varroa mites in the phoretic stage and mites under capped cells. However, to be truly effective, a treatment should result in a significant long term reduction in the varroa population levels. Such a change in mite numbers should be detectable as a decrease in mite/day drop in the treated colonies compared to control colonies. The average mite/day in treated hives spiked at treatment but steadily decreased over time, Fig. 3.

At 24 days post treatment, which represents a full cycle of brood production, the average mite drop levels differed significantly among all categories ($P < 0.001$. $H=16.3$, $DF = 3$ Kruskal-Wallis test). Subsequent analysis using Dunn's test revealed significant differences between each of the treated categories and the control ($P < 0.05$ in all cases) but no differences among the 3 formic acid groups ($P > 0.05$ in all cases). The average mite/day drop among treated hives was 11.6 mites/day while the control hives average was 70.8 mites/day.

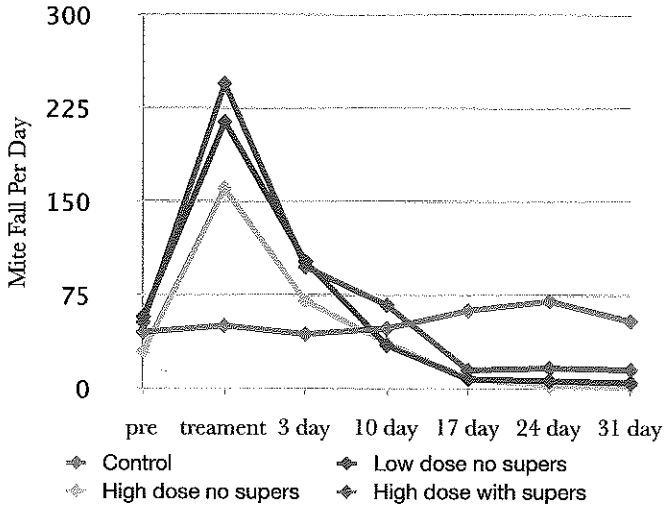
### Product application and temperature concerns

The ambient temperature at the apiary during the three day application period was very variable; the average daily ambient temperature was 26.17 °C (79.1 °F) with a range of 23 to 37 °C (73.4 to 98.6 °F). These ambient temperatures would be problematic for other formic acid treatments including Mite Away II, which is also a NOD Apiary product. Mite Away II, as is the case for other fumigants including thymol, has the potential to cause large brood damage if applied during high ambient temperatures. According to the EPA, Mite Away II should not be applied if the ambient temperature exceeds 26.1 °C (79 °F). Mite Away II should also be removed from the hives if the ambient temperature goes above 27.7 °C (82 °F) during treatment to avoid brood kill and/or absconding.

The average daily temperatures in our trials were on the upper end of the Mite Away II use recommendation and the peak temperature of 37 °C (98 °F) experienced during one of the three treatment days was well above the proper use of Mite Away II. However, in spite of the high ambient temperatures and high humidity levels (average relative humidity was 82.6 %, (range: 52 to 96 %) registered during the application period, the treated colonies suffered very little adult and/or brood mortality with this new product (see Colony Effects).

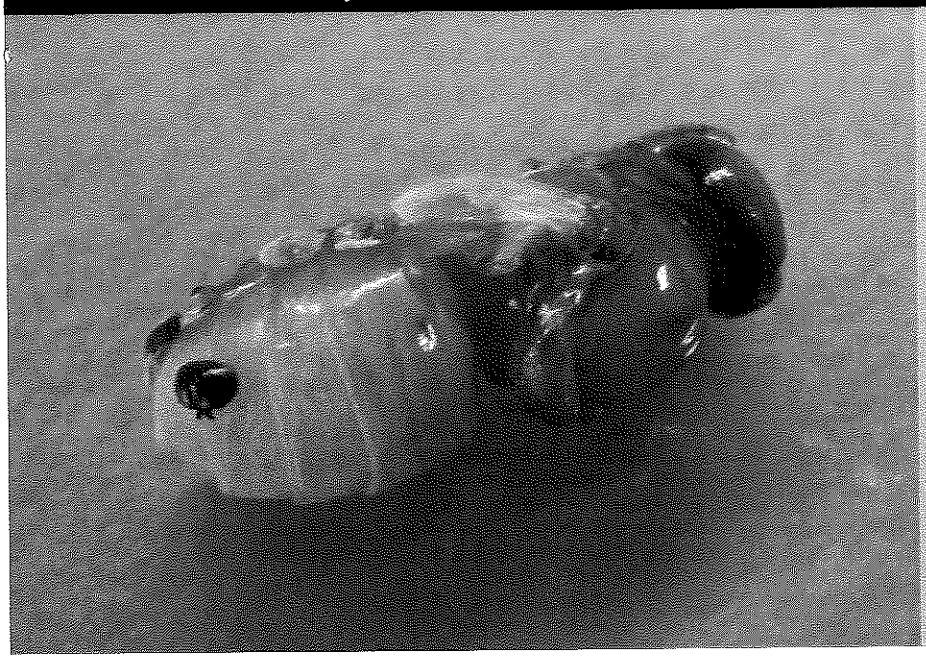
The core hive temperatures and humidity are among the most important factors that regulate the release rate of the new product. The core hive temperatures were on average higher, but more constant, than the ambient temperatures. The average core temperature of the hives was 35.6 °C, (96.1 °F, range 31 to 37 °C or 87.8 to 98.6 °F) and the average relative humidity was 68.8% (range 60 to 80.4%). The NOD formic acid flash strips released their fumigant vapors in response to the microclimatic conditions within the hive. The formic acid strips lost approximately 40% of their total weight during the 72 hours inside the hive, and the product matrix had become drier and more brittle even under the elevated humidity in the field.

**Figure 3: Mite fall levels over time**




| | |
|---|---|
| Maximum mite fall/ hive 31 days post treatment | 5806 mites |
| Maximum mite fall/ hive at treatment | 2188 mites |
| Percent infestation of drone brood in control hives 31 days post treatment (n=6) | 39 percent |
| Percent infestation of drone brood in treated hives 31 days post treatment (n=5) | 8 percent |

Mite population levels in Hawaiian honeybee colonies can be very high compared to the levels in the mainland USA and Europe. For example 4 colonies experienced mite drops of over 3000 mites during the formic acid trial. This apparent "tolerance" is probably a temporary condition that could change as the viral diseases spread and become more virulent. Nevertheless, the ability of formic acid to kill mites under capped cells contributes greatly to the overall decrease in mite population levels. The drone infestation levels was significantly lower in treated versus control hives at 31 days post treatment (Mann-Whitney U-test, $p=0.004$).



RESULTS

## Mite life cycle and treatment efficacy

Because varroa mites spend a portion of their adult life in a phoretic phase, feeding on the adult bees, and then switch to a reproductive phase, laying eggs inside bee cells, it is difficult to successfully target both stages with a single type of treatment.

Most varroa treatments usually target phoretic mites, and consequently need to be applied for over 21 days in order to kill any emerging adult mites. Formic acid however, has the advantage to work well under capped cells.

## Mite mortality under capped cells

We collected drone brood samples from four treated colonies and one control colony at the time the formic acid strips were removed from the hives. Dissection of those samples revealed that adult mites under capped cells suffered an increase in mortality compared to untreated colonies. Fifty percent of the adult female mites were found dead within the capped cells of treated colonies (103 cells sampled, total of 164 mites in the sampled cells), while all the adult mites under capped cells in control hives were alive upon dissection of the comb (total of 18 cells sampled, total of 25 adult female mites.

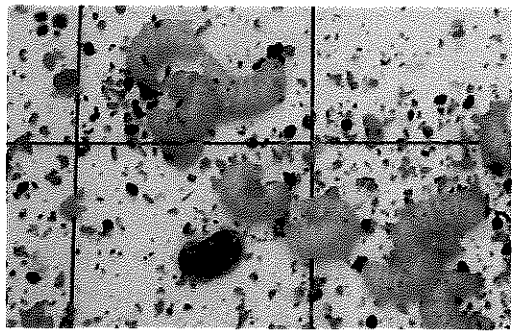
Adult males also suffered an increase in mortality in treated colonies compared to control colonies; male death was 71% and 33% respectively. The death of the male mite has particularly serious consequences for the surviving daughter mites since mating and fertilization only take place within the cell. Premature male death is likely to result in a large number of unfertilized adult females that will not be able to contribute to the next mite generation. Preliminary data also suggests that immature mites of both sexes suffered an even higher mortality than adult mites. Over 90% of all young mites were found dead following treatment, while only 18 % of juveniles were dead in the control colonies. It is possible that the tough exoskeleton of the adult mites protected them from damage during the formic acid treatment but that the younger stages were more susceptible to the vapors.

Mite mortality within capped cells is an important element in varroa control. Although a large proportion of mites died in the cells, it is still unclear whether the adult mites that survived were damaged by the treatment and if their reproductive ability may have been compromised. The University of Hawaii plans to conduct a smaller scale study of mite mortality under capped cells.

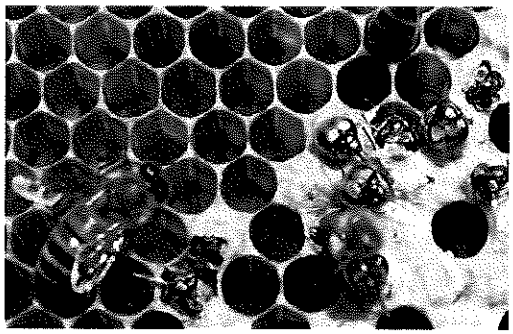
## Formic acid residues in honey

Varroa control can be difficult because any chemical substance applied to a honeybee colony has the potential to leave a residue in honey and/or wax. Honey samples from control and treated hives were analyzed for changes in formic acid content during the trial. Results indicate that there was no significant increase in formic acid levels 72 hours after product application. Treated colonies had an average of 923 parts per million (ppm) pre treatment and 856 ppm at treatment. Control colonies had average pre treatment levels of 805 ppm compared to 839 ppm at the 72 hours mark. The short term treatment duration of the new NOD product application combined with the recent results of the formic acid residues in Hawaii suggest that withdrawal of the honey supers is not needed, and greatly facilitates the management efforts.

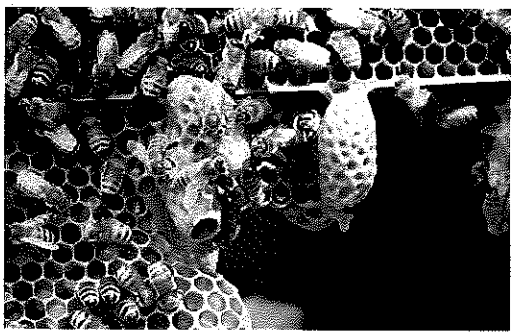
# RESULTS
## Colony effects



The observed brood damaged was minimal. About 2/3 of the hives removed larvae from the cells during the 3 day treatment. The discarded larvae fell onto the sticky boards and were counted. The average number of larvae discarded was about 22 larvae/hive (range 0-130), which is relatively small.



A few bees that were emerging right during the treatment were suffocated by the vapors (emerging brood burn). Bees under capped cells were apparently unharmed by the application and the overall levels of brood disruption were negligible.



There were no adverse effects on queens derived from the treatments. Following treatment with formic acid the queens were located visually and/ or egg laying was observed in all the treated hives.

In fact, new queens hatched during the 3 day treatment period, as indicated by the destruction by the virgin queen of the younger, unopened queen cells.

The UH Honeybee Varroa Project Oahu team included Dr. Mark Wright, Dr. Ethel Villalobos, Scott Nikaido, and Tyler Ito. The NOD Apiary team included David and Mary VanderDussen. We would like to thank the beekeepers who so generously provided experimental colonies and logistical support for this trial: Dennis Takata, Rhea McWilliams, Howard McGinnis, George Hudes, and Charlie Reppun. Funding for this project was provided by the Hawaii Department of Agriculture.

Report produced by Ethel M. Villalobos, Ph.D. Plant and Environmental Protection Sciences, CTAHR, August 2009.

Design by Jonathan Wright. Photos by S. Nikaido, E. Villalobos, and J. Wright.

**LINDA LINGLE**
Governor

**SANDRA LEE KUNIMOTO**
Chairperson, Board of Agriculture

**DUANE K. OKAMOTO**
Deputy to the Chairperson

State of Hawaii
**DEPARTMENT OF AGRICULTURE**
Pesticides Branch
1428 South King Street
Honolulu, Hawaii 96814-2512
Phone: (808) 973-9401 Fax: (808) 973-9418

October 19, 2009

Document Processing Desk (SLN)
Office of Pesticide Programs (7504P)
U.S. Environmental Protection Agency
Room S-4900, One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202-4501

Dear Product Manager:

Enclosed is a special local need (24(c)) registration for the use of Mite-Away Quick Strips (MAQS), unregistered, for the treatment of honey bee colonies infested with varroa mites (*Varroa destructor*).

Registered alternatives include Apistan Anti-Varroa Mite Strip (Fluvalinate), CheckMite+ Bee Hive Pest Control Strip (coumaphos), Apiguard (thymol), ApiLife Var (thymol), Mite-Away II (formic acid) and Sucrocide Concentrate (sucrose octanoate esters).

Issues including early signs of resistance, chemical residues in wax (which adversely affects queen production), lack of a non-reproductive period in the tropics, and high ambient temperatures and humidity, significantly limit use of the alternatives.

A signed copy of the "Application for / Notification of State Registration of a Pesticide...", and the Hawaii Department of Agriculture "ACCEPTED with Comments" stamped label is enclosed. The EPA SLN Number assigned to this registration is HI-090002.

Should you have any questions, please contact Lance Kobashigawa, Pesticide Specialist, at (808) 973-9411.

Sincerely,

for Lyle Wong, Ph.D.
Administrator, Plant Industry Division

LW:lk
[0902epa]

c: Glenda Dugan (CED5), EPA Region 9

# Mite-Away Quick Strips™

For Use Only in the State of Hawaii.

**For Treatment of Honey Bees Infested with Varroa Mites**

| **Active Ingredient** | |
|---|---|
| **Formic Acid** | **48.4%** |
| **Other Ingredients** | **51.6%** |
| **Total** | **100.0%** |

**KEEP OUT OF REACH OF CHILDREN**

ACCEPTED

OCT 2 6 2009

Under Hawaii Pesticides Law as supplement to Product No. 8589.2

**DANGER-POISON –**
**CORROSIVE TO SKIN AND EYES**

**PELIGRO-Si usted no entiende la etiqueta, busque a alguien para que se la explique a usted en detalle.**

**SEE SIDE PANEL FOR FIRST AID STATEMENT**

**Registration for Special Local Need – Hawaii**
**EPA SLN No. HI-090002**

**EPA Establishment Number: 075710-CAN-001**

**Best before Date:**

**NOD Apiary Products USA Inc.**
P.O. Box 142, 231 Frankford Rd,
Stirling, Ontario, Canada K0K 3E0
www.miteaway.com

**Product Information Telephone: (866)483-2929** **Product of Canada**

**Net Contents: TBD**
**XXX** Mite-Away Quick Strips™, XXX treatments

Mite-Away Quick Strips™ – For treatment of honey bee colonies infested with varroa mites.
Mite-Away Quick Strips™ requires a single application of **two strips per treatment** to reduce the number of varroa mites for one season.

## Precautionary Statements

| FIRST AID STATEMENT | |
|---|---|
| **If inhaled** | •Move person to fresh air.<br>•If a person is not breathing, call 911 or an ambulance, then give artificial respiration, preferably by mouth-to-mouth, if possible.<br>•Call a poison control center or doctor for further treatment advice. |
| **If on skin or clothing** | •Take off contaminated clothing<br>•Rinse skin immediately with plenty of water for 15-20 minutes.<br>•Call a poison control center or doctor for treatment advice |
| **If in eyes** | •Hold eye open and rinse slowly and gently with water for 15-20 minutes.<br>•Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye.<br>•Call a poison control center or doctor for treatment advice |
| **If swallowed** | •Call a poison control center or doctor for further treatment advice.<br>•Have a person sip a glass of water if able to swallow.<br>•Do not induce vomiting unless told to do so by the poison control center or doctor.<br>•Do not give anything by mouth to an unconscious person |
| **Poison Control Center HOT LINE NUMBER 1-800-222-1222** | |
| Have the product container with you when calling a poison control center or doctor, or going for treatment. | |
| NOTE TO PHYSICIAN: Probable mucosal damage may contraindicate the use of gastric lavage. | |

### HAZARDOUS TO HUMANS AND DOMESTIC ANIMALS

### DANGER – PELIGRO

Fatal if inhaled, absorbed through the skin, or swallowed. Do not breathe dust, vapor or spray mist. Corrosive. Causes skin burns and irreversible eye damage. Avoid contact with skin, eyes, or clothing. Wash skin thoroughly with soap and water after handling and before eating, drinking, chewing gum or using tobacco. Remove and wash contaminated clothing before reuse.

### ENVIRONMENTAL HAZARDS

For terrestrial use: Do not apply directly to water or to areas where surface water is present or to intertidal areas below the mean high water mark. Do not contaminate water when disposing of used container, wash water or rinsate.

### PHYSICAL OR CHEMICAL HAZARDS

Corrosive – Do not allow product to contact metal surfaces. Do not place, even briefly, on metallic hive covers. Store unused product in original container.

**Handler Personal Protective Equipment (PPE):** Applicators and other handlers must wear coveralls over a long-sleeved shirt, long pants, socks and shoes, acid resistant gloves (PVC, neoprene, or nitrile), and protective eyewear. Wear a respirator with an organic-vapor removing cartridge with a prefilter approved for pesticides (MSHA/NIOSH approval number prefix TC-23C), or a canister approved for pesticides (MSHA/NIOSH approval number prefix TC-14G), or a NIOSH approved respirator with an organic vapor (OV) cartridge or canister with any N, R, P or HE prefilter. Clean or replace PPE at end of each day's work period. Rinse off pesticides at rest breaks. Follow the manufacturer's instructions for cleaning/maintaining PPE. If no such instructions for washables exist, use detergent and hot water. Keep and wash PPE separately from other laundry.

**User Safety Recommendations:**

Users should remove Personal Protective Equipment/clothing immediately if pesticide gets inside. Wash thoroughly and put on clean clothing. Replace chemical gloves if punctured or stretched.

Have water readily available should skin or eye contact occur.

Only use out of doors, stand upwind of product. Use caution when opening the container, especially in warm weather.

## Agricultural Use Requirements

Use this product only in accordance with its labeling and with the Worker Protection Standard, 40 CFR part 170. This standard contains requirements for the protection of agricultural workers on farms, forests, nurseries, and greenhouses, and handlers of agricultural pesticides. It contains requirements for training, decontamination, notification, and emergency assistance. It also contains specific instructions and exceptions pertaining to the statements on this label about personal protective equipment (PPE), notification to workers, and restricted-entry interval. The requirements in this box apply to uses of this product that are covered by the Worker Protection Standard.

Re-entry interval: Do not enter or allow worker entry into treated area (bee hive) during the restricted entry interval (REI) of 72 hrs.

Appropriate PPE as listed must be worn for re-entry into the treated area (bee hive) after the 72 hour REI and within the remaining 7 days.
PPE required for entry into treated bee hives (that is permitted under the Worker Protection Standard and that involves contact with anything that has been treated), is:
Wear coveralls over a long-sleeved shirt, long pants, socks and shoes, acid resistant gloves (PVC, neoprene or nitrile), and protective eyewear. Wear a respirator with an organic-vapor removing cartridge with a pre-filter approved for pesticides (MSHA/NIOSH approval number prefix TC-23C), or a canister approved for pesticides (MSHA/NIOSH approval number prefix TC-14G), or a NIOSH approved respirator with an organic vapor (OV) cartridge or canister with any N, R, P or HE pre-filter.

Notify workers of the application by warning them orally and by posting warning signs at entrances to treated bee yards. Posting in the bee yard should read as follows: *The hives in this bee yard have been treated with Mite-Away Quick Strips™. Treatment was applied: Date and time. Do not open the bee hives in this yard until after the 72 hour REI unless wearing PPE as stated on the product label at any time within the 7 day treatment period.*

## DIRECTIONS FOR USE - Mite-Away Quick Strips™

It is a violation of Federal law to use this product in a manner inconsistent with its labeling. Do not apply this product in a way that will contact workers or other persons, either directly or through drift. Only protected handlers may be in the area during application. For any requirements specific to your State, consult the Agency in your State responsible for pesticide regulation.

WHEN TO TREAT: Use Mite-Away Quick Strips™ as part of an Integrated Pest Management (IPM) program. Treat only if treatment thresholds are exceeded. Treatment period is for this product is 7 days. Treatment ends at day 7.
When treatment levels are reached, use Mite-Away Quick Strips™ for single or double brood-chamber, standard Langstroth equipment honeybee hives, honeybee colony cluster covering a minimum of 6 brood frames. Outside daytime temperature highs should be between 70 - 92°F on days of application. Excessive temperatures (> 95°F) during the first three days of treatment can cause excessive brood mortality and absconding.
Brood mortality may occur in the initial stage of treatment. Overall colony health is not expected to be affected, with brood rearing returning to normal by the end of treatment. **Treatment of smaller colonies than those listed on the label will result in excessive brood mortality and even in colony mortality.**
APPLICATION Disturb the colony activity as little as possible during the application process. Remove the Mite-Away Quick Strips™ from the outer pouch. For hives with single brood chambers lay two strips across the top bars of the frames of the brood chambers, staggering them so they lay flat and across the full width of the hive body, with approximately 2 inches between strips and 4 inches between the ends of the brood chamber and the outer edges of the strips. For hives with two brood chambers place the strips as described above on the frame top bars of the lower hive body, so the strips are in-between the brood chambers. Put on honey supers, if a honey flow is anticipated. The active ingredient dissipates after 3 days however, do not disturb the colony for 7 days to allow it to recover from any side-effects that may have occurred. Spent strips need not be removed after

treatment.
The bottom hive entrance needs to be fully open for the entire duration of treatment. Other entrances should be sealed. Entrance reducers MUST be removed to prevent excessive damage to the colonies. Treat all bee colonies in the apiary at the same time. Allow a minimum of one month between applications.

**STORAGE AND DISPOSAL**

Do not contaminate water, food, or feed by storage or disposal.

STORAGE: Store in original container in a cool, dry, and well-ventilated area away from sulphuric acid, oxidizing agents, and sources of ignition and away from the reach of children. Avoid heat, sparks, and open flames. Do not eat, drink or smoke in areas of use or storage. Use caution when opening the container, especially in warm weather, i.e.: open outdoors and stay downwind. Keep separate to prevent cross-contamination of other pesticides, fertilizer, food, or feed.

FOR DISPOSAL OF UNUSED, UNWANTED, OR DAMAGED PRODUCT: CONTACT THE MANUFACTURER or the National Pesticide Information Center at 800-858-7378 (www.npic.orst.edu).

CONTAINER DISPOSAL: If empty, triple rinse the container. Place in trash or offer for recycling if available. If partly filled: Call your local solid waste agency or (800-858-7378/www.npic.orst.edu) for disposal instructions.
PLASTIC BAG (OUTER WRAP) DISPOSAL: Rinse or air dry empty outer pouch, wearing PPE, and then dispose in a sanitary landfill or by incineration, or, if allowed by state and local authorities, by burning. If burned, stay out of smoke.

Pesticide wastes are acutely hazardous. Improper disposal of excess pesticide, spray mixture, or rinsate is a violation of Federal Law. If these wastes cannot be disposed of by use according to label instructions, contact your State Pesticide or Environmental Control Agency, or the Hazardous Waste Representative at the nearest EPA Regional Office for guidance.

## Warranty Statement:

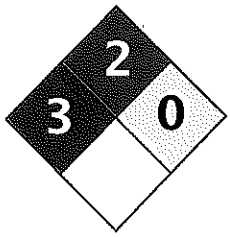
To the extent allowable by state law, the seller shall not be liable for any damages when the product is misused or not used in accordance with label directions.

This special local need registration label is valid until October 18, 2014 or until otherwise revised, amended, cancelled or suspended.

Issue date: October 19, 2009

Expiration date: October 18, 2014

ScienceLab.com
Chemicals & Laboratory Equipment




| | |
|---|---|
| Health | 3 |
| Fire | 2 |
| Reactivity | 0 |
| Personal Protection | |

# Material Safety Data Sheet
# Formic acid, 85%, F.C.C MSDS

## Section 1: Chemical Product and Company Identification

**Product Name:** Formic acid, 85%, F.C.C

**Catalog Codes:** SLF1387

**CAS#:** Mixture.

**RTECS:** Not applicable.

**TSCA:** TSCA 8(b) inventory: Formic acid; Water

**CI#:** Not applicable.

**Synonym:** Formic Acid, 85%

**Chemical Name:** Not applicable.

**Chemical Formula:** Not applicable.

**Contact Information:**

**Sciencelab.com, Inc.**
14025 Smith Rd.
Houston, Texas 77396

US Sales: **1-800-901-7247**
International Sales: **1-281-441-4400**

Order Online: ScienceLab.com

**CHEMTREC (24HR Emergency Telephone), call:**
1-800-424-9300

**International CHEMTREC, call:** 1-703-527-3887

**For non-emergency assistance, call:** 1-281-441-4400

## Section 2: Composition and Information on Ingredients

**Composition:**

| Name | CAS # | % by Weight |
|---|---|---|
| Formic acid | 64-18-6 | 85 |
| Water | 7732-18-5 | 15 |

**Toxicological Data on Ingredients:** Formic acid: ORAL (LD50): Acute: 700 mg/kg [Mouse]. 1100 mg/kg [Rat]. 4000 mg/kg [Dog]. VAPOR (LC50): Acute: 6200 mg/m 0.25 hours [Mouse].

## Section 3: Hazards Identification

**Potential Acute Health Effects:**
Very hazardous in case of skin contact (irritant), of eye contact (irritant, corrosive), of ingestion, . Hazardous in case of skin contact (corrosive, permeator). Slightly hazardous in case of inhalation (lung sensitizer). Non-corrosive for lungs. Liquid or spray mist may produce tissue damage particularly on mucous membranes of eyes, mouth and respiratory tract. Skin contact may produce burns. Inhalation of the spray mist may produce severe irritation of respiratory tract, characterized by coughing, choking, or shortness of breath. Inflammation of the eye is characterized by redness, watering, and itching. Skin inflammation is characterized by itching, scaling, reddening, or, occasionally, blistering.

**Potential Chronic Health Effects:**
Slightly hazardous in case of skin contact (sensitizer).
CARCINOGENIC EFFECTS: Not available.

MUTAGENIC EFFECTS: Mutagenic for mammalian somatic cells. [Formic acid]. Mutagenic for bacteria and/or yeast. [Formic acid].
TERATOGENIC EFFECTS: Not available.
DEVELOPMENTAL TOXICITY: Not available.
The substance may be toxic to kidneys, liver, upper respiratory tract, skin, eyes, central nervous system (CNS). Repeated or prolonged exposure to the substance can produce target organs damage. Repeated or prolonged contact with spray mist may produce chronic eye irritation and severe skin irritation. Repeated or prolonged exposure to spray mist may produce respiratory tract irritation leading to frequent attacks of bronchial infection.

## Section 4: First Aid Measures

**Eye Contact:**
Check for and remove any contact lenses. In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Cold water may be used. Get medical attention immediately.

**Skin Contact:**
In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Cover the irritated skin with an emollient. Cold water may be used.Wash clothing before reuse. Thoroughly clean shoes before reuse. Get medical attention immediately.

**Serious Skin Contact:**
Wash with a disinfectant soap and cover the contaminated skin with an anti-bacterial cream. Seek immediate medical attention.

**Inhalation:**
If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention immediately.

**Serious Inhalation:**
Evacuate the victim to a safe area as soon as possible. Loosen tight clothing such as a collar, tie, belt or waistband. If breathing is difficult, administer oxygen. If the victim is not breathing, perform mouth-to-mouth resuscitation. WARNING: It may be hazardous to the person providing aid to give mouth-to-mouth resuscitation when the inhaled material is toxic, infectious or corrosive. Seek medical attention.

**Ingestion:**
Do NOT induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. If large quantities of this material are swallowed, call a physician immediately. Loosen tight clothing such as a collar, tie, belt or waistband.

**Serious Ingestion:** Not available.

## Section 5: Fire and Explosion Data

**Flammability of the Product:** Combustible.

**Auto-Ignition Temperature:** The lowest known value is 539°C (1002.2°F) (Formic acid).

**Flash Points:** The lowest known value is OPEN CUP: 69°C (156.2°F). (Formic acid)

**Flammable Limits:** The greatest known range is LOWER: 18% UPPER: 57% (Formic acid)

**Products of Combustion:** These products are carbon oxides (CO, CO2).

**Fire Hazards in Presence of Various Substances:**
Flammable in presence of open flames and sparks, of heat.
Slightly flammable to flammable in presence of metals.
Non-flammable in presence of shocks, of oxidizing materials, of reducing materials, of combustible materials, of organic materials, of acids, of alkalis, of moisture.

**Explosion Hazards in Presence of Various Substances:**

Explosive in presence of oxidizing materials.
Slightly explosive in presence of organic materials, of metals.
Non-explosive in presence of open flames and sparks, of shocks.

**Fire Fighting Media and Instructions:**
SMALL FIRE: Use DRY chemical powder.
LARGE FIRE: Use water spray, fog or foam. Do not use water jet.

**Special Remarks on Fire Hazards:**
Decomposes more rapidly under fire conditions, forming carbon monoxide.
Aluminum reduces formic acid (itself a reductant) with incandescence. (Formic acid)

**Special Remarks on Explosion Hazards:**
Formic acid forms explosive reactions with the following: Furfuryl alcohol, Hydrogen Peroxide + organic matter; Nitromethane, P2O5, Thallic nitrate trihydrate +vanillin, and oxidizing agents
Explosive decompositon of Formic Acid on clean nickel. (Formic acid)

## Section 6: Accidental Release Measures

**Small Spill:**
Dilute with water and mop up, or absorb with an inert dry material and place in an appropriate waste disposal container. If necessary: Neutralize the residue with a dilute solution of sodium carbonate.

**Large Spill:**
Combustible material. Corrosive liquid.
Keep away from heat. Keep away from sources of ignition. Stop leak if without risk. Absorb with DRY earth, sand or other non-combustible material. Do not get water inside container. Do not touch spilled material. Use water spray curtain to divert vapor drift. Prevent entry into sewers, basements or confined areas; dike if needed. Call for assistance on disposal. Neutralize the residue with a dilute solution of sodium carbonate. Be careful that the product is not present at a concentration level above TLV. Check TLV on the MSDS and with local authorities.

## Section 7: Handling and Storage

**Precautions:**
Keep locked up.. Keep container dry. Keep away from heat. Keep away from sources of ignition. Ground all equipment containing material. Do not ingest. Do not breathe gas/fumes/ vapor/spray. Never add water to this product. In case of insufficient ventilation, wear suitable respiratory equipment. If ingested, seek medical advice immediately and show the container or the label. Avoid contact with skin and eyes. Keep away from incompatibles such as oxidizing agents, organic materials, acids, alkalis.

**Storage:**
Keep container in a cool, well-ventilated area. Keep container tightly closed and sealed until ready for use. Avoid all possible sources of ignition (spark or flame).

## Section 8: Exposure Controls/Personal Protection

**Engineering Controls:**
Provide exhaust ventilation or other engineering controls to keep the airborne concentrations of vapors below their respective threshold limit value. Ensure that eyewash stations and safety showers are proximal to the work-station location.

**Personal Protection:**
Face shield. Full suit. Vapor respirator. Be sure to use an approved/certified respirator or equivalent. Gloves. Boots.

**Personal Protection in Case of a Large Spill:**
Splash goggles. Full suit. Vapor respirator. Boots. Gloves. A self contained breathing apparatus should be

used to avoid inhalation of the product. Suggested protective clothing might not be sufficient; consult a specialist BEFORE handling this product.

**Exposure Limits:**
Formic acid
TWA: 5 STEL: 10 (ppm) from ACGIH (TLV) [United States] [1999]
TWA: 9 (mg/m3) from NIOSH
TWA: 5 (ppm) from NIOSH
TWA: 9 (mg/m3) from OSHA (PEL) [United States]
TWA: 5 (ppm) from OSHA (PEL) [United States]
TWA: 5 (ppm) [United Kingdom (UK)]
TWA: 9.3 (mg/m3) [United Kingdom (UK)]3
Consult local authorities for acceptable exposure limits.

## Section 9: Physical and Chemical Properties

**Physical state and appearance:** Liquid.

**Odor:** Pungent. Penetrating. Benzaldehyde-like

**Taste:** Sour

**Molecular Weight:** Not applicable.

**Color:** Clear Colorless.

**pH (1% soln/water):** Acidic.

**Boiling Point:** The lowest known value is 100°C (212°F) (Water). Weighted average: 100.67°C (213.2°F)

**Melting Point:** May start to solidify at 8.4°C (47.1°F) based on data for: Formic acid.

**Critical Temperature:** Not available.

**Specific Gravity:** Weighted average: 1.21 (Water = 1)

**Vapor Pressure:** The highest known value is 4.7 kPa (@ 20°C) (Formic acid). Weighted average: 4.6 kPa (@ 20°C)

**Vapor Density:** The highest known value is 1.59 (Air = 1) (Formic acid). Weighted average: 1.55 (Air = 1)

**Volatility:** Not available.

**Odor Threshold:** The highest known value is 0.625 ppm (Formic acid)

**Water/Oil Dist. Coeff.:** Not available.

**Ionicity (in Water):** Not available.

**Dispersion Properties:** See solubility in water, diethyl ether, acetone.

**Solubility:**
Easily soluble in acetone.
Soluble in cold water, hot water, diethyl ether.

## Section 10: Stability and Reactivity Data

**Stability:** The product is stable.

**Instability Temperature:** Not available.

**Conditions of Instability:** Heat, ignition sources, incompatible materials

**Incompatibility with various substances:**
Highly reactive with oxidizing agents.
Reactive with organic materials, metals, acids, alkalis.

**Corrosivity:**
Highly corrosive in presence of copper.
Corrosive in presence of stainless steel(304).
Non-corrosive in presence of glass, of aluminum, of stainless steel(316).

**Special Remarks on Reactivity:**
Formic acid is a strong reducing agent. Decomposes slowly during storage! Vent Container At Least Monthly. Formic acid may react with alkalies and oxidizing materials such as peroxides, nitric acid, and chromic acid. It is also incompatible with concentrated Sulfluric Acid, Nitromethane, finely powdered metals, permanganates, strong bases, oxidizing agents. (Formic acid)

**Special Remarks on Corrosivity:** Corrosive to metals

**Polymerization:** Will not occur.

## Section 11: Toxicological Information

**Routes of Entry:** Absorbed through skin. Dermal contact. Eye contact. Inhalation. Ingestion.

**Toxicity to Animals:** Acute oral toxicity (LD50): 729 mg/kg (Mouse) (Calculated value for the mixture).

**Chronic Effects on Humans:**
MUTAGENIC EFFECTS: Mutagenic for mammalian somatic cells. [Formic acid]. Mutagenic for bacteria and/or yeast. [Formic acid].
May cause damage to the following organs: kidneys, liver, upper respiratory tract, skin, eyes, central nervous system (CNS).

**Other Toxic Effects on Humans:**
Extremely hazardous in case of inhalation (lung corrosive).
Very hazardous in case of skin contact (irritant), of eye contact (corrosive), of ingestion, .
Hazardous in case of skin contact (corrosive, permeator).

**Special Remarks on Toxicity to Animals:** Not available.

**Special Remarks on Chronic Effects on Humans:** May affect genetic material (mutagenic)

**Special Remarks on other Toxic Effects on Humans:**
Acute Potential Health Effects:
Skin: Corrosive. Causes skin irritation and burns . Absorbed through the skin. May cause erythema and blistering.
Eyes: Corrosive. Causes eye irritation and burns. Lachrymator. May cause corneal edema, ulceration and scaring. Vapors may cause itching, burning and swelling of the eyes.
Inhalation: Affects respiration and causes respiratory tract irritation and burns. Vapors may afect behavior (brain) and sense organs and cause dizziness, and nausea. May also affect the urinary system and liver
Ingestion: May be harmful if swallowed. Causes digestive tract irritation and burns with abdominal pain, vomiting, and possible death. May product corrosive ulceration and bleeding, and necrosis of the gastrointestinal tract. May also affect the cardiovascular system, urinary system, blood, behavior, and metabolism.
Chronic Potential Health Effects: Prolonged or repeated skin contact may cause dermatitis. Mah cause liver and kidney damage. Effects may be delayed. Laboratory experiments have resulted in mutagenic effects.

## Section 12: Ecological Information

**Ecotoxicity:** Not available.

**BOD5 and COD:** Not available.

**Products of Biodegradation:**
Possibly hazardous short term degradation products are not likely. However, long term degradation products may arise.

**Toxicity of the Products of Biodegradation:** The products of degradation are less toxic than the product itself.

**Special Remarks on the Products of Biodegradation:** Not available.

## Section 13: Disposal Considerations

**Waste Disposal:**
Waste must be disposed of in accordance with federal, state and local environmental control regulations.

## Section 14: Transport Information

**DOT Classification:** Class 8: Corrosive material

**Identification:** : Formic acid (Formic acid) UNNA: 1779 PG: II

**Special Provisions for Transport:** Not available.

## Section 15: Other Regulatory Information

**Federal and State Regulations:**
New York release reporting list: Formic acid
Rhode Island RTK hazardous substances: Formic acid
Pennsylvania RTK: Formic acid
Florida: Formic acid
Minnesota: Formic acid
Massachusetts RTK: Formic acid
New Jersey: Formic acid
TSCA 8(b) inventory: Formic acid; Water
SARA 313 toxic chemical notification and release reporting: Formic acid
CERCLA: Hazardous substances.: Formic acid: 5000 lbs. (2268 kg);

**Other Regulations:** OSHA: Hazardous by definition of Hazard Communication Standard (29 CFR 1910.1200).

**Other Classifications:**

**WHMIS (Canada):**
CLASS B-3: Combustible liquid with a flash point between 37.8°C (100°F) and 93.3°C (200°F).
CLASS E: Corrosive liquid.

**DSCL (EEC):**

**HMIS (U.S.A.):**

**Health Hazard:** 3

**Fire Hazard:** 2

**Reactivity:** 0

**Personal Protection:**

**National Fire Protection Association (U.S.A.):**

**Health:** 3

**Flammability:** 2

**Reactivity:** 0

**Specific hazard:**

**Protective Equipment:**
Gloves.
Full suit.
Vapor respirator. Be sure to use an approved/certified respirator or equivalent. Wear appropriate respirator when ventilation is inadequate.
Face shield.

## Section 16: Other Information

**References:** Not available.

**Other Special Considerations:** Not available.

**Created:** 10/09/2005 05:35 PM

**Last Updated:** 11/06/2008 12:00 PM

**National Toxicology Program**
Toxicity Report Series
Number 19

# NTP Technical Report on Toxicity Studies of

# Formic Acid

**(CAS No: 64-18-6)**

## Administered by Inhalation to F344/N Rats and B6C3F$_1$ Mice

**Morrow Thompson, DVM, PhD, Study Scientist**
**National Toxicology Program**
**Post Office Box 12233**
**Research Triangle Park, NC 27709**

**NIH Publication 92-3342**
**July 1992**

**United States Department of Health and Human Services**
**Public Health Service**
**National Institutes of Health**

NOTE TO THE READER

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July, 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from the Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this toxicity study report were performed under the direction of the NIEHS and were conducted in compliance with NTP chemical health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals.

These studies are designed and conducted to characterize and evaluate the toxicologic potential of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection per se is not an indicator of a chemical's toxic potential. Single copies of this Report are available without charge while supplies last from the NTP Public Information Office, NIEHS, P.O. Box 12233, Research Triangle Park, NC 27709 (919-541-3991).

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**National Institutes of Health**

# CONTRIBUTORS

The NTP report on the toxicity studies of formic acid is based primarily on the 2-week studies that began in August, 1987, and ended in September, 1987, and the 13-week studies that began in December, 1987, and ended in March, 1988, at Battelle Northwest Laboratories.

**National Toxicology Program**
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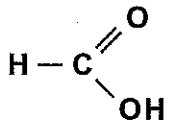
# Formic Acid



**Molecular Formula:** HCOOH
**CAS No.:** 64-18-6
**Molecular Weight:** 46

**Synonyms:** Aminic Acid, Formylic Acid, Methanoic Acid, Hydrogen Carboxylic Acid

## ABSTRACT

Formic acid occurs in a variety of plants and fruits, mammalian tissues, and insect venoms. It is used industrially in preparing a variety of drugs, dyes, and chemicals; as a decalcifier; and in leather tanning. Formic acid also is an environmental contaminant of air and water and has been identified as the toxic intermediate (formate) in methanol poisoning. Two- and 13-week toxicity studies of formic acid were conducted in male and female F344/N rats and B6C3F$_1$ mice by whole body inhalation exposure to formic acid vapors. In addition, *in vitro* genetic toxicity studies were performed with *Salmonella typhimurium,* with or without metabolic activation. Formic acid was not mutagenic in this assay.

In 2-week studies, groups of 5 F344/N rats and 5 B6C3F$_1$ mice of each sex were exposed to formic acid for 6 hours a day, 5 days a week, at concentrations of 0, 31, 62.5, 125, 250, or 500 ppm. Deaths occurred in animals exposed to 500 ppm (rats and mice) and 250 ppm (1 female mouse). Microscopic lesions in the respiratory and olfactory epithelia occurred in rats and mice exposed to 62.5 ppm and higher concentrations, with the severity related to the exposure concentration. The lesions consisted of squamous metaplasia, necrosis, and inflammation. Exposures had minimal or no effects on coagulation times, blood pH and electrolytes, or on concentrations and activities of urine analytes in rats during the 2-week studies.

In 13-week studies, groups of 10 animals of each species and sex were exposed to formic acid at concentrations of 0, 8, 16, 32, 64, and 128 ppm for 6 hours a day, 5 days a week. Two mice, 1 male and 1 female, died in the 128 ppm groups. Body weight gains were significantly decreased in mice exposed to 64 and 128 ppm formic acid. Microscopic changes in rats and mice ranged from minimal to mild in severity and generally were limited to animals in the 128 ppm groups. Lesions

related to exposure to formic acid consisted of squamous metaplasia and degeneration of the respiratory and olfactory epithelia, respectively. Hematologic and serum biochemical changes at interim and terminal time points were minimal to mild and, generally, were consistent with hemoconcentration.

Overall, the effects of formic acid were consistent with those of irritant chemicals administered by inhalation exposure. The no-observed-adverse-effect level (NOAEL) for respiratory injury was 32 ppm in rats and mice. There was no significant evidence of systemic toxicity in these studies.

# PEER REVIEW

## Peer Review Panel

The members of the Peer Review Panel who evaluated the draft report on the toxicity studies on formic acid on July 10, 1991, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel members act to determine if the design and conditions of the NTP studies were appropriate and to ensure that the toxicity study report fully and clearly presents the experimental results and conclusions.

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## Summary of Peer Review Comments

On July 9 and 10, 1991, the Technical Reports Review Subcommittee of the Board of Scientific Counselors for the National Toxicology Program met in Research Triangle Park, NC, to review the draft technical report on toxicity studies of formic acid

Dr. M. Thompson, NIEHS, introduced the short-term toxicity study report by reviewing the natural occurrences and uses of formic acid, the experimental design, and the results.

Dr. Carlson, a principal reviewer, said the study was well done. He asked at what point after the 2-week study the blood pH was determined, noting that adidosis is an important problem with the acute toxicity of methanol through its metabolism to formate. Dr. Thompson said pH was determined the day following the last exposure to formic acid. Dr. Carlson also asked that a rationale be given for administering the chemical by inhalation. Dr. Thompson said that formic acid had been nominated for study because of its structural relationship with formaldehyde and because inhalation is an important route of exposure for humans.

Dr. Klaassen, a second principal reviewer, said the study was performed well. He said he was concerned that the report may over-emphasize that rodent data on formic acid exposure may not be applicable to humans. He said the localized toxic effects observed might be very relevant for humans. Dr. Thompson said the lack of a systemic toxic effect in rats may be related to their resistance to formate toxicity, and that this was the reason for the emphasis. Dr. Klaassen agreed but said that the possible similarity in local toxic effects among rodents and humans should be made more clear.

Dr. Zeise questioned the NOAEL reported in the study (32 ppm), noting a reported olfactory epithelial lesion in a male rat at 32 ppm in the 13-week study. Dr. M. Elwell, NIEHS, said the olfactory degeneration was a minimal change and that it was difficult to cite it as a treatment effect.

After discussion of editorial matters, the panel agreed to accept to report, with the suggested changes.

# INTRODUCTION

## Physical Properties, Occurrence, Production, Uses, and Exposure

Formic acid, a colorless, highly caustic liquid with a pungent odor, has a melting point of 8.4°C, a boiling point of 100.7°C, a density of 1.220 g/cm$^3$ at 20°C, and a vapor pressure of 400 mm Hg at 24°C. Vapor and liquid forms of formic acid are flammable, especially at temperatures greater than 69°C. Formic acid is miscible with water, alcohol, and ether, soluble in benzene and toluene, and very soluble in acetone. It can react as an acid and as an aldehyde (Wagner, 1980). Under normal storage conditions formic acid can deteriorate by dehydration, dehydrogenation, or through a bimolecular redox reaction.

Formic acid, first described by Fisher in 1670 in the products resulting from the distillation of red ants (Windholz, 1983), occurs in both natural and man-made sources in the environment. A constituent of ant, wasp, and bee venom, formic acid also occurs in mammalian muscle tissue, sweat, and urine. It is found in plants, such as in the needles of the Douglas fir, and in unripened grapes, peaches, raspberries, strawberries, petitgrain lemon, and in bitter orange (SRI, 1981). It also is present in many foods (Gley, 1967), e.g., fruits (20 - 40 ppm), fruit juices (30 - 100 ppm), fruit syrups (650 - 1630 ppm), honey (20 - 2000 ppm), wines (1 - 340 ppm), coffee, roasted (1350 - 2200 ppm), coffee, extracts (2000 - 7700 ppm), evaporated milk (30 - 400 ppm), and cheese (20 - 200 ppm) (Tracor Jitco, 1974).

An air and water pollutant, formic acid has been measured at concentrations ranging from 4 to 72 ppm in the atmosphere. It has been identified in river and surface water, in unfinished industrial waste water, and in municipal sewage and final municipal discharge water at concentrations ranging from approximately 10 to 80,000 µg /L (SRI, 1981). Other sources of formic acid include forest fires, lacquer manufacturing, trash and plastic burning, and tobacco smoke. Thermal degradation of polyethylene during manufacturing may result in the release of formic acid and various aldehydes into the atmosphere (Zitting and Savolainen, 1980).

Formic acid is synthesized industrially by heating carbon monoxide and sodium hydroxide under pressure, then treating the resulting sodium formate with sulfuric acid. It also is prepared by acid hydrolysis of methyl formate and as a by-product in the manufacture of acetaldehyde and formaldehyde (Wagner, 1980). U.S. production and import figures for formic acid between 1976 and 1979 ranged from 2.3 to 3.1 x $10^7$ kg/year to 3.7 x $10^3$ to 1.0 x $10^6$ kg/year, respectively.

Formic acid is used extensively as a decalcifier, as an acidulating agent in textile dying and finishing, and in leather tanning (Wagner, 1980). It also is used in the preparation of organic esters and in the manufacture of drugs, dyes, insecticides, and refrigerants. Other functions include use as a mold inhibitor on grain and silage, as a solvent for perfumes, as a plasticizer for vinyl resin, and as a coagulator for latex. Trace amounts of formate esters are used in the formulation of fragrances and flavors, especially fruit and honey mixtures (Wagner, 1980; Sittig, 1985).

The Threshold Limit Value (TWA) for exposure to airborne formic acid is 5 ppm or 9 mg/m$^3$ and is based on irritation to the respiratory tract (ACGIH, 1986). No criteria for a permissible ambient water standard have been set; however, the EPA has suggested a level of 124 mg/L (Sittig, 1985).

According to NIOSH (1980), approximately 533,799 workers were exposed occupationally to formic acid during the period 1972 to 1974. Public exposure results from the consumption of various food products such as fruit juices, honey, wines, coffee, unripened grapes, and strawberries. Human exposure also occurs through exposure to the atmosphere and water because of the compound's presence in the environment.

## Human Toxicity

Formic acid is caustic and can cause damage to skin, eyes, and mucosal surfaces (International Labour Office, 1983). Chronic absorption has been reported to cause albuminuria and hematuria (Windholz, 1983). Inhalation of formic acid results in rhinitis, cough, bronchitis, and dyspnea; ingestion causes corrosion and necrosis of the mucous membranes of the mouth, throat, esophagus, and stomach. Extensive exposure can produce depression of the central nervous system, severe metabolic acidosis, and nephropathy (Seiler *et al.*, 1988). Swallowing formic acid has resulted in a number of cases of severe poisoning and death (Sittig, 1985). In some cases of fatal poisoning, hematuria and anuria develop, and the patient may die from uremia, circulatory failure, or pneumonia. Air levels of formic acid were measured at 15 ppm in a textile plant in which workers were complaining of nausea (ACGIH, 1986).

## Animal Studies

Oral $LD_{50}$ values for formic acid in rats range from 1100 to 1850 mg/kg. $LD_{50}$ values for mice range from 700 to 1100 mg/kg for oral, 940 mg/kg for i.p., and 145 mg/kg for i.v. administration. Reported $LD_{50}$ values from inhalation studies were 15 g/m$^3$/15 minutes for rats and 6.2 g/m$^3$/15 minutes for mice. Clinical signs included respiratory distress and unidentified behavioral changes. An oral $LD_{50}$ value of 4000 mg/kg has been reported for dogs (Malorny, 1969; NIOSH, 1985; Sax and Lewis, 1989).

Reductions in body weight gains at the higher doses were the only effects seen in studies in which young rats (~40 g, strain unspecified) were administered formic acid in the diet or drinking water at levels of 0.5 or 1.0% for 6 weeks, or in which rats received 8 to 360 mg/kg formic acid in drinking water for 2 to 27 weeks (Clayton and Clayton, 1981). Hypochromic anemia and a mild lymphocytosis developed in rats receiving formic acid in the diet. It has been reported that the survival of offspring obtained from female rats administered 1.0% formic acid in drinking water for up to 7 months was reduced by 50 to 67% (Tracor Jitco, 1974). No other references to studies of reproductive toxicity or teratogenicity of formic acid were located.

There were small but statistically significant changes in activities of drug metabolizing as well as in other enzymes in liver, kidney, and brain of male Wistar rats exposed to 20 ppm formic acid vapor for 3 or 8 days, 6 hours per day (Zitting and Savolainen, 1980). Concentrations of glutathione in brain, liver, and kidney were decreased in exposed rats; activities of lysosomal acid proteinase in brain and ethoxycoumarin deethylase in liver were increased, while those of cytochrome P-450 in kidney were decreased compared to controls.

## Disposition and Metabolism

Formic acid is absorbed from the gastrointestinal tract, lungs, intact skin, and urinary bladder. The absorbed compound is oxidized to $CO_2$ and $H_2O$, partly excreted unchanged in the urine, and partly metabolized in tissues. The main site of oxidation is the liver, although intestinal mucosa, lungs, kidney, and spleen also contribute. Oxidation of formate occurs by folate-dependent and catalase-peroxidative mechanisms. In rats, monkeys, and human beings, half-lives of sodium formate in blood are 12 - 23, 31 - 51, and 55 minutes, respectively (Clay *et al.*, 1975; McMartin *et al.*, 1977; Rietbrock *et al.*, 1971). The rate of formate oxidation to $CO_2$ in monkeys was markedly lower than that in rats. Although the rate of oxidation was dose-dependent in both species, metabolism in monkeys proceded at a rate approximately one-half that measured in rats (McMartin *et al.*, 1977). Rates of formate oxidation were 40 mg/kg/hr in monkeys, 300 mg/kg/hr in mice, and 78 mg/kg/hr in rats. Excretion of formic acid also is influenced by the amount administered; 8% - 9% was excreted unchanged by dogs given a 1 g oral dose as compared to 65% excreted by dogs given a 5 g dose (Tracor Jitco, 1974).

Methyl chloride and formaldehyde are metabolized to formate, which is metabolized further by folic acid-dependent pathways; then they either are incorporated into tissue macromolecules or oxidized to $CO_2$ and $H_2O$ (Kornbrust *et al.*, 1982; Mashford *et al.*, 1982). In addition, methanol toxicity is associated with accumulation of formate (McMartin *et al.*, 1977; Clay *et al.*, 1975; Martin-Amat *et al.*, 1978). Methanol is rapidly metabolized to formaldehyde primarily by the catalase-peroxidative system in rats, although the alcohol dehydrogenase and microsomal cytochrome P-450 enzyme systems are active in the rat, guinea pig, and rabbit (Tephly *et al.*, 1964; Mannering *et al.*, 1969; Teschke *et al.*, 1975; Dalvi and Townsend, 1976). Only human beings and monkeys rely primarily on the alcohol dehydrogenase system. Formaldehyde is rapidly metabolized to formate by formaldehyde dehydrogenase (Uotila and Koivusalo, 1974a; 1974b) and does not accumulate in rats or monkeys after dosing with methanol (Makar and Tephly, 1977; McMartin *et al.*, 1977, 1979). Formaldehyde metabolism to formate also can occur by various aldehyde dehydrogenases. In primates and rodents, oxidation of formate to $CO_2$ is accomplished primarily by folate-dependent metabolism (McMartin *et al.*, 1979; Palese and Tephly, 1975). Although of lesser importance, this step also can be catalyzed by catalase peroxidative oxidation in rodents. Urinary excretion of formate may be an important route of elimination in folate-deficient rodents (Smith and Taylor, 1982).

The susceptibility of a species to methanol toxicity is inversely related to its capacity for tetrahydrofolate-dependent oxidation of formate (McMartin *et al.*, 1977). Tetrahydrofolate levels in the liver of monkeys are 60% of those in rats and are thought to account for a 50% lower maximal rate of formate oxidation in monkeys as compared to rats (Black *et al.*, 1985). Inhibition of methionine synthetase, an enzyme important in the synthesis of tetrahydrofolate, by administering nitrous oxide ($N_2O$) or feeding a folate-deficient diet, renders rats susceptible to methanol toxicity (Eells *et al.*, 1981; Makar and Tephly, 1977). In a recent study, activities of the enzyme, 10-formyl-tetrahydrofolate dehydrogenase, which catalyzes the oxidation of 10-formyl-tetrahydrofolate to $CO_2$ and tetrahydrofolate, were compared in human and rat liver (Johlin *et al.*, 1989). The finding that properties of the enzymes were similar, but that the activity was lower in human than in rat liver, may be an additional factor contributing to the accumulation of high levels of formate during

methanol metabolism in humans as compared to rats. On a quantitative basis, this indicates that humans should be more susceptible to formate toxicity than the rat.

## Chronic Toxicity/Carcinogenicity

Formic acid and several other chemicals were tested for tumor promotion in a dermal exposure study. An 8% formic acid and water solution was applied on both sides of both ears of male Swiss mice (Frei and Stephens, 1968). The animals' ears were pretreated with one application of 1.5% 7, 12-dimethylbenz(a)anthracene (DMBA). The formic acid solution was applied to the ears twice a week for 20 weeks with the first application occurring 1 week after treatment with DMBA. Animals treated with formic acid had hyperplasia and epidermal thickening at incidences similar to or below those of controls; formic acid was concluded not to be a tumor promoter in this study.

## Genetic Toxicity

Formic acid (10 - 3333 µg /plate) was not mutagenic in *Salmonella typhimurium* strains TA100, TA1535, TA97, or TA98, with or without S9 (Zeiger *et al.,* 1992). It also did not induce sister-chromatid exchanges, with or without S9, in hamster V79 cells treated with a maximum dose of 2mM (Basler *et al.,* 1985).

Positive results were reported for formic acid in tests for induction of sex-linked recessive lethal mutations in germ cells of male Drosophila (Stumm-Tegethoff, 1969) and in tests for induction of chromosomal aberrations in Chinese hamster ovary cells (Morita *et al.,* 1990). However, in both these investigations, it was noted that neutralizing the acidic pH of the test solution or nutrient medium, or increasing the buffering power of the solutions, eliminated the mutagenic responses. It was concluded that formic acid by itself is non-mutagenic, but that testing at concentrations which produce non-physiological pH levels results in a "false positive" response due to perturbations in the test system.

## Study Rationale and Design

Formic acid was nominated as part of an air pollutant class study by the National Cancer Institute, based on its high potential for human exposure (~533,800 workers exposed occupationally from 1972 to 1974); its structural relationship to formaldehyde, a known nasal carcinogen in rats (Swenberg *et al.,* 1980); and because of the lack of information concerning the toxicity and carcinogenicity of the chemical. In response, the NTP conducted 2-week and 13-week inhalation studies using male and female B6C3F1 mice and Fischer 344/N rats. Mutagenicity studies were conducted in *Salmonella typhimurium,* using buffered solutions of formic acid to reduce the potential for false positive responses.

# MATERIALS AND METHODS

## Procurement and Characterization of Formic Acid

Formic acid was obtained from BASF Wyandotte Corporation (Parsippany, NJ). Cumulative analytical data for the chemical indicated a purity of approximately 95%, with approximately 5% water as the only significant contaminant. The infrared, ultraviolet/visible, and nuclear magnetic resonance spectra were consistent with the structure of formic acid and available literature references. Elemental analysis results for carbon and hydrogen agreed with theoretical values, corrected for the water content. Karl Fischer titrimetry indicated 4.87 ± 0.07% water. Functional group titration indicated a purity of 94.9 ± 0.3%. Gas chromatography by 1 system (10% SP-1000/1% $H_3PO_4$) resolved 1 peak. A second gas chromatography system (15% SP-1220/1% $H_3PO_4$) resolved a major peak and an impurity peak with an area of 2.0% relative to the area of the major peak. This impurity was identified tentatively as water, on the basis of retention time with water-spiked samples.

The chemical was administered to animals by inhalation exposure. Formaldehyde was determined as a possible degradation product of formic acid in the exposure chambers, and determinations of formaldehyde levels were made in occupied and unoccupied chambers containing 8 and 128 ppm formic acid as well as in the formic acid distribution line (~2500 ppm formic acid). Grab samples, using gas sampling tubes packed with N-benzylethanolamine coated on a solid support, were collected and subsequently analyzed by gas chromatography for the 3-benzyloxazolidine derivative. In addition, the stability of formic acid in the generator reservoir over extended time periods was investigated. These studies revealed no evidence of significant degradation of formic acid. The amount of formaldehyde collected was less than 0.1% of the collected amount of formic acid in all samples that were taken.

Repeated purity analyses of samples taken from the formic acid generator indicated that formic acid did not decompose in the generator reservoir over a period of at least 29 days. No significant discrepancies in formic acid purity were observed in any of these analyses.

## Vapor Generation

Animals were exposed and maintained in 1.7 $m^3$ inhalation chambers, commercially produced by Harford Systems, Inc., (Aberdeen, MD). Bulk liquid formic acid was contained in an 8-liter, stainless steel, nitrogen-blanketed reservoir confined within a vented steel cabinet. As the formic acid was used, nitrogen replaced the formic acid and served to exclude $O_2$ from the reservoir. Liquid to be vaporized was pumped from the reservoir to a vaporizer by a micrometering pump which was constructed of materials compatible with formic acid. All liquid delivery tubing was constructed of Teflon®.

The vaporizer consisted of a stainless steel cylinder covered with a glass fiber wick from which the liquid was vaporized. An 80-watt heater and 2 temperature sensing elements were incorporated within the cylinder. One sensing element was connected to a remote temperature controller

allowing vaporizer temperatures of up to 120°C, with control to better than ± 0.5°C. The vaporizer was operated at approximately 97 ± 5°C. The other sensing element was connected to a digital temperature readout device. Output from this device was recorded at periodic intervals. The cylindrical vaporizer was positioned in the fresh-air duct leading directly to the vapor distribution manifold.

After flash vaporization, the vapor entered a short distribution manifold where the individual delivery lines carried a metered amount of vapor to each exposure chamber. A constant concentration of formic acid vapor, approximately 2300 ppm, was maintained in the distribution duct. Dilution air was conditioned to room temperature at approximately 50% relative humidity and was filtered by HEPA and charcoal filters. Vacuum transvector pumps, located at the chamber end of each vapor delivery line, generated negative pressure to draw the formic acid vapor from the distribution manifold through fine metering valves to the chambers. The high-concentration vapor was diluted by conditioned air to achieve the required target concentration immediately before entry of the vapor into the chambers.

The time after the start of exposure for the concentration to reach 90% of the final stable concentration in the chamber (T90) and the time after the termination of generation for the vapor concentration to decay to 10% of the stable concentration (T10) were determined. T90, with and without animals in the chamber with a flow of 15 CFM, was 10 and 27 minutes, respectively. T10, with and without animals, was 10 - 11 minutes and 40 - 100 minutes, respectively.

## Concentration Monitoring

A Foxboro Miran 980 infrared spectrometer (The Foxboro Co., Foxboro, MA) with a 20-meter, variable-pathlength, heated (~80°C) gas cell was used to monitor the exposure chambers, control chamber, exposure room, an on-line standard of formic acid vapor, and a pure nitrogen source. All locations were monitored approximately once every 40 minutes. The infrared cell was set to a 9.75-meter pathlength; the analytical wavelength for formic acid was 9.050 microns. Water was measured at 6.535 microns to allow a small correction for the absorbance of water vapor at the analytical wavelength for formic acid. A reference measurement was performed at 4.045 microns to correct for instrument drift.

The on-line monitor was calibrated by comparing monitor readings with GC analyses of grab samples collected from the exposure chambers at the time of the readings. The limit of detection and limit of quantification for the on-line monitor were determined at an average chamber relative humidity of 33-51%. The practical detection limit was 0.36 ± 0.10 ppm, with a practical quantification limit of 0.68 ± 0.10 ppm. During the 2-week and the 13-week studies, at least 96% and 91%, respectively, of the measured concentrations for each chamber were within ± 10% of the target concentration.

## Toxicity Study Design

Male and female Fischer 344/N rats and B6C3F$_1$ mice used in these studies were produced under strict barrier conditions at Taconic Farms (Germantown, NY). Animals were the progeny of defined microflora-associated parents that were transferred from isolators to barrier-maintained rooms. Rats and mice were shipped to the study laboratory at 4 weeks of age, quarantined at the study

laboratory for 11-13 days, and placed on study at 6-7 weeks of age. Blood samples were collected and the sera analyzed for viral titers from 5 animals per sex and species at study start and at termination in the 13-week studies. Data from 5 viral screens performed in rats and 12 viral screens performed in mice (Boorman *et al.*, 1986; Rao *et al.*, 1989; 1989a) showed that there were no positive antibody titers.

During the acclimation period, animals were randomly assigned to test groups using body weight as the blocking variable. Once exposure began, the animals were housed continuously in exposure chambers with chamber doors closed, except during animal husbandry procedures. Pelleted NIH-07 feed (Zeigler Bros., Inc., Gardners, PA) was available to animals at all times except during the daily exposure period, when feed was removed. Drinking water was available *ad libitum*.

Groups of 5 rats and 5 mice of each sex were administered formic acid by inhalation exposure for 12 days, 6 hours per day +T90/day (30 minutes), 5 days per week. Exposure concentrations for rats and mice were 0, 31, 62.5, 125, 250, or 500 ppm. After the third day of exposure, rats were removed from the inhalation chambers and placed in metabolism cages for a 16-hour collection of urine. Animals had access to water but not food. Urine collection tubes were placed in ice/water baths. Measurements included volume, pH, concentrations of glucose and protein, and activities of aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), and alkaline phosphatase (AP). Rats were returned to the inhalation chamber after the collection period.

On the day following the end of the 2-week exposure period, blood samples were collected from rats for determination of pH, concentrations of serum electrolytes, and coagulation times. Immediately prior to termination, rats were anesthetized with an i.p. injection of sodium pentobarbital, and blood samples were collected into plastic syringes from the lumbar aorta. For measurement of blood pH, samples were quickly transferred to a capillary tube containing heparin, and a Radiometer BME-33 blood pH instrument (Radiometer America, Inc., Westlake, OH) was used for the analyses. For determination of concentrations of sodium, potassium, chloride, and total $CO_2$, blood samples were placed into tubes devoid of an anticoagulant, and serum was harvested. Instrumentation Laboratories instruments, Models 442 and 446 (Instrumentation Laboratories, Lexington, MA), were used for these determinations. For determination of prothrombin and partial thromboplastin times, samples were placed in 3.8% sodium citrate and tests were performed using Dade reagents and a BBL Fibrometer (BBL Microbiology Systems, Cockeysville, MD). Necropsy examinations were performed on all animals (rats and mice). Weights were determined for the liver, thymus, right kidney, right testis, heart, and lungs. The following tissues in all control and treated animals were trimmed, embedded, stained with H&E, and examined microscopically: gross lesions, larynx, lungs and attached tracheobronchial lymph nodes, nasal cavity, and trachea. Further details are outlined in Table 1.

In the 13-week studies, rats and mice in groups of 10 per sex were exposed to formic acid vapor by whole body exposure at target concentrations of 0, 8, 16, 32, 64, and 128 ppm for 6 hours +T90/day (30 minutes), 5 days/week. Ten additional male and female rats per group were included for clinical pathology studies; clinical observations were recorded daily. Body weights were recorded at study start, at weekly intervals, and at the end of the studies. Organ weights were determined for the thymus, heart, right kidney, lungs, liver, and right testis.

Clinical pathology studies were performed on the additional rats on days 3 and 23, and on core study rats at study termination. Animals were anesthetized with 70% $CO_2$:30% $O_2$ and bled from the retroorbital sinus using heparinized microcapillary tubes. Samples for hematologic analyses (~0.50 mL) were collected in tubes containing dry potassium EDTA, gently mixed, and held at room temperature until analyzed. Blood samples for serum analyses (~0.75 mL) were collected into tubes containing a separator gel but without an anticoagulant. These samples were allowed to clot at room temperature for approximately 30 minutes and centrifuged at 5000 g for 10 minutes; the serum then was harvested for biochemical analyses.

Automated hematologic analyses were performed using an Ortho ELT-8/ds hematology system (Ortho Diagnostics Systems, Inc., Westwood, NJ). The following variables were measured (or calculated): erythrocyte, leukocyte, and platelet counts, hemoglobin (HGB) concentration, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Smears of peripheral blood were stained with Wrights stain and examined microscopically. Leukocyte differentials were determined on 100 cells; absolute counts of individual leukocytes were calculated based on the total leukocyte count and the relative number. Reticulocytes were stained by mixing equal volumes of whole blood with new methylene blue stain for 20 minutes. Relative numbers of reticulocytes, determined by microscopic examination of approximately 1000 erythrocytes, were converted to absolute counts based on the total erythrocyte count.

Biochemical analyses were performed using an Abbott VP analyzer (Abbott Laboratories, North Chicago, Ill.) The following assays were performed using reagents and methods provided by the manufacturer: urea nitrogen (UN), creatinine, total protein, albumin, alanine aminotransferase (ALT), alkaline phosphatase (AP), creatine kinase (CK), and amylase. For determination of activity of sorbitol dehydrogenase (SDH), a reagent kit was obtained from Sigma Chemical Company (St. Louis, MO) and adapted for the Abbott VP. For determination of total bile acids, a reagent kit was obtained from Nyegaard Diagnostica (Enzabile, Oslo, Norway), and concentrations were measured as an end-point reaction using a recording spectrophotometer.

A complete necropsy was performed on all animals. Organs and tissues were examined for gross lesions. Tissues were fixed in 10% neutral buffered formalin. Tissues for microscopic evaluation were dehydrated and embedded in paraffin, sectioned at approximately 5 microns, and stained with hematoxylin and eosin, and examined microscopically.

Upon completion of the histologic evaluation by the laboratory pathologist, the slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were sent to an independent pathology laboratory for quality assessment, and the results were reviewed and evaluated by the NTP Pathology Working Group (PWG). The final diagnoses represent a consensus of contractor pathologists and the PWG. Details of these review procedures have been described by Maronpot and Boorman (1982) and Boorman *et al.* (1985).

## Reproductive Toxicity

Sperm morphology and vaginal cytology examinations were performed for rats and mice administered formic acid at 0, 8, 32, and 128 ppm in the 13-week study. To screen for potential reproductive toxicity, epididymal sperm motility was evaluated at necropsy, and vaginal cytology was evaluated on animals during the 2 weeks just preceding necropsy, using procedures outlined by Morrissey *et al.* (1988). For the 12 days prior to sacrifice, females were subject to vaginal lavage with saline. The aspirated cells were air-dried onto slides, stained with Toluidine Blue O, and cover slipped. The relative preponderance of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were used to identify the stages of the estrual cycle.

Sperm motility was evaluated at necropsy as follows: The left epididymis was removed and quickly weighed; the cauda epididymis was removed at the junction of the vas deferens and the corpus epididymis, then weighed. Warm (37°C) Tyrodes buffer (mice) or test yolk buffer (rats) was applied to two pre-warmed slides, and a small cut made in the distal cauda epididymis. The sperm that extruded from the epididymis were dispersed throughout the solution, cover slipped, and counted immediately on a warmed microscope stage. Two independent observers counted the number of moving and non-moving sperm in 5 fields of 30 sperm or less per field. After sperm sampling for motility evaluation, the cauda was placed in phosphate buffered saline (PBS), gently chopped with a razor blade, and allowed to sit for 15 min. The remaining clumps of tissue were removed and the solution mixed gently and heat-fixed at 65°C. Sperm density was subsequently determined using a hemocytometer.

To quantify spermatogenesis, the left testis was weighed, frozen and stored. After thawing, testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the testis in PBS containing 10% DMSO. Homogenization-resistant spermatid nuclei were enumerated using a hemocytometer; the data were expressed as spermatid heads per total testis and per gram of testis.

## Genetic Toxicology

### Experimental Protocol

Testing was performed as described by Haworth *et al.* (1983). Formic acid was incubated with the *Salmonella typhimurium* tester strains TA97, TA98, TA100, and TA1535, either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley or Syrian hamster liver) for 20 minutes prior to the addition of soft agar supplemented with L-histidine and D-biotin, and subsequently plated on minimal glucose agar plates. Incubation continued for an additional 48 hours.

Each test consisted of triplicate plates of concurrent positive and negative controls and at least 5 doses of test chemical. High dose was limited by toxicity and did not exceed 3.33 mg/plate. All positive assays were repeated under the conditions which elicited the positive response.

A positive response was defined in this assay as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any single strain/activation combination. An equivocal

response was defined as an increase in revertants which was neither dose-related, reproducible, or of sufficient magnitude to support a determination of mutagenicity. A negative response was obtained when no increase in revertant colonies was observed following chemical treatment.

## Statistical Methods

Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data, which are approximately normally distributed, were analyzed using the parametric multiple comparisons procedures of Williams (1971, 1972) and Dunnett (1955). Clinical chemistry and hematology data, which typically have skewed distributions, were analyzed using the nonparametric multiple comparisons methods of Shirley (1977) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of dose-response trends and to determine whether a trend-sensitive test (Williams, Shirley) was more appropriate for pairwise comparisons than a test capable of detecting departures from monotonic dose-response (Dunnett, Dunn). If the P-value from Jonckheere's test was greater than or equal to 0.10, Dunn's or Dunnett's test was used rather than Shirley's or Williams' test.

The outlier test of Dixon and Massey (1951) was employed to detect extreme values. No value selected by the outlier test was eliminated unless it was at least twice the next largest value or at most half of the next smallest value.

Because the vaginal cytology data are proportions (the proportion of the observation period that an animal was in a given estrous state), an arcsine transformation was used to bring the data into closer conformance with normality assumptions. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for the simultaneous equality of measurements across dose levels.

## Quality Assurance

The 13-week toxicity studies of formic acid were performed in compliance with FDA Good Laboratory Practices regulations (21 CFR 58). The Quality Assurance Unit of Battelle Northwest Laboratories performed audits and inspections of protocols, procedures, data, and reports throughout the course of the studies. The operations of the Quality Assurance Unit were monitored by the NTP.

## TABLE 1 Experimental Design and Materials and Methods in the 14-Day and 13-Week Inhalation Studies of Formic Acid

| | |
|---|---|
| **Study Laboratory** | Battelle Pacific Northwest Laboratories |
| **Study Dates** | 14-Day Studies: August -- September, 1987<br>14-Day Dosed Feed Studies: December 1987 -- March, 1988 |
| **Strain and Species** | F344/N rats; B6C3F$_1$ mice |
| **Animal Source** | Taconic Farms, Inc., Germantown, NY |
| **Chemical Source** | BASF Wyandotte Corporation (Parsippany, NJ). |
| **Size of Study Groups** | 14-Day Studies:<br>5/sex/group of each species. Animals were individually caged.<br>13-Week Studies:<br>Mice--10/sex/group; rats--20/sex/group (10 core study and 10 for clinical pathology). Animals were individually caged. |
| **Method of Animal Distribution** | Animals assigned to dosed and control groups by computer-generated tables of random numbers, using body weight as a blocking variable. |
| **Route of Administration** | Whole body inhalation |
| **Exposure Concentrations** | 14-Day Studies: 0, 31, 62.5, 125, 250, and 500 ppm<br>13-Week Studies: 0, 8, 32, 64, and 128 ppm |
| **Diet** | NIH-07 available *ad libitum* except during exposure periods |
| **Animal Room Environment** | Temp--75 ± 3°F; relative humidity--55 ± 15%; fluorescent light 12 h/d;<br>15 ± 3 air changes/h. |
| **Time Held Before Study** | 14-Day Studies: Rats-11 d; Mice-12 d<br>13-Week Studies: Rats-12 d, Mice-13 d |
| **Age When Placed on Study** | 14-Day and 13-Week Studies: 6 wks (7 wks for mice in 13-week studies). |
| **Age When Killed** | 14-Day Studies: 8 wks<br>13-Week Studies: Rats-19 wks; Mice-20 wks |
| **Type and Frequency of Observation** | 14-Day Studies:<br>Observed 2 x d for mortality/moribundity; 2 x d each exposure day for clinical signs of toxicity; weighed on days 1, 8, and at necropsy.<br>13-Week Dosed Feed Studies:<br>Observed 2 x d for mortality/moribundity; body weights and clinical observations measured weekly and at necropsy. |

**Necropsy and Histologic Examinations (14-day studies)**

Necropsy was performed on all animals. The following tissues were examined microscopically: lungs, trachea, larynx, bronchial lymph nodes, nose (three transverse sections), and all gross lesions from all treated and control animals. Urinalysis, coagulation, serum chemistry were performed at day 3 and at termination.

**Necropsy and Histologic Examinations (13-week studies)**

Necropsy was performed on all animals. The following tissues were examined microscopically from all control and high dose groups: adrenal glands, brain, bronchial lymph nodes, cecum, colon, duodenum, epididymis/seminal vesicles/ prostate/testes or ovaries/uterus, esophagus, eyes (if grossly abnormal), femur (including marrow), gallbladder (mice), gross lesions and tissue masses with regional lymph nodes, heart, ileum, jejunum, kidneys, larynx, liver, lungs with mainstem bronchi, mammary gland and adjacent skin, mandibular and mesenteric lymph nodes, mediastinal lymph nodes, nasal cavity and turbinates, pancreas, parathyroid glands, pharynx (if grossly abnormal), pituitary gland, preputial /clitoral glands (rats), rectum, salivary glands, spinal cord and sciatic nerve (if neurologic signs present), spleen, stomach (including forestomach and glandular stomach), thigh muscle, thymus, thyroid gland, trachea, and urinary bladder. In addition to all gross lesions, the following tissues were examined in all other dose groups: rats--nose (three transverse sections), lung, larynx, trachea, bronchial and mediastinal lymph nodes; mice--nose (three transverse sections). Organ weights (to the nearest mg) were obtained from all core study animals and include: liver, thymus, right kidney, right testis, heart and lungs. Hematologic and serum chemical analyses were performed; sperm morphology and vaginal cytology were evaluated in rats and mice exposed to 0, 8, 32, and 128 ppm.

# RESULTS

## 2-Week Studies in Rats

One female and 3 male rats in the 500 ppm exposure groups died on day 10 of exposure (Table 2). Final body weights were significantly lower in male rats exposed to 250 and 500 ppm formic acid and in female rats exposed to 500 ppm, compared to control animals (Table 2). Exposure-related clinical signs were limited to the 250 and 500 ppm dose groups and were consistent with effects typically seen with respiratory irritants. Clinical signs noted included nasal discharge, increased preening, hypoactivity, and labored breathing. Male and female rats among the highest dose group developed corneal opacity.

**TABLE 2 Survival and Weight Gain of F344/N Rats in the 2-Week Inhalation Studies of Formic Acid**

| Exposure Concentration (ppm) | Survival[a] | Mean Body Weight (grams) | | | Final Weight Relative to Controls (%)[c] |
|---|---|---|---|---|---|
| | | Initial | Final | Change[b] | |
| **MALE** | | | | | |
| 0.0 | 5/5 | 119 | 177 | 58 | |
| 31.0 | 5/5 | 119 | 179 | 60 | 101 |
| 62.5 | 5/5 | 117 | 175 | 58 | 99 |
| 125.0 | 5/5 | 119 | 172 | 53 | 97 |
| 250.0 | 5/5 | 119 | 162 | 43 | 92 |
| 500.0 | 2/5 | 120 | 135 | 15 | 76 |
| **FEMALE** | | | | | |
| 0.0 | 5/5 | 94 | 127 | 33 | |
| 31.0 | 5/5 | 93 | 126 | 33 | 99 |
| 62.5 | 5/5 | 92 | 131 | 39 | 103 |
| 125.0 | 5/5 | 91 | 123 | 32 | 97 |
| 250.0 | 5/5 | 94 | 118 | 24 | 93 |
| 500.0 | 4/5 | 94 | 96 | 2 | 76 |

a Number surviving at 14 days/number of animals per dose group.

b Mean weight change of the animals in each dose group.

c (Dosed group mean/control group mean) x 100.

Effects of treatment on blood pH and concentrations of serum electrolytes were unremarkable. A mild, statistically significant increase occurred in concentrations of sodium in female rats in the highest exposure group (500 ppm). There did not appear to be any consistent effect of formic acid exposure on coagulation tests, as results of assays of prothrombin time and activated partial thromboplastin time did not differ among the groups (not shown). Results of urinalyses (performed after exposure day 3) indicated a reduction in 16-hour urine volumes in males and females exposed to 250 ppm and in males exposed to 500 ppm; urine specific gravity was variably increased in exposed males and females, and correlated with reductions in urine volume. Similarly, concentrations of glucose and protein, and activities of AST, GGT and AP were increased, but when corrected for total 16-hour excretion, they were unchanged from controls.

At necropsy, exposure-related gross lesions consisted of dried exudate around the external (anterior) nares in 3 males and 3 females from the 500 ppm exposure groups. Although cloudiness of the cornea was observed clinically at this exposure concentration during the course of the study,

corneal opacity was identified in only 1 male rat at the time of necropsy. This corneal change was characterized microscopically by a very minimal inflammatory cell infiltrate (neutrophils).

Both absolute and relative thymus weights were significantly less (as much as 50% in male and female rats exposed to 500 ppm) compared to controls. There were no differences in other absolute organ weights between exposed and control animals. The relative weights of the kidney in males and females, and of the heart in females, were increased significantly in high-dose animals compared to controls; however, the group mean body weights of these animals were lower than controls, which contributed to these differences.

Histopathologic lesions related to formic acid exposure in the upper respiratory tract were similar in nature, and dose-related in incidence and severity, in male and female rats exposed at concentrations of 62.5 ppm or higher (Table 3). With exposure concentrations of 125 ppm or higher, lesions occurred in the respiratory and olfactory epithelium in the anterior (Level I) and mid portion (Level II) of the nasal cavity. Lesions were most severe at the 500 ppm exposure level; squamous metaplasia and necrosis of the respiratory and olfactory epithelium were present in all rats. An inflammatory cell (neutrophils) infiltrate was present in the mucosa, and exudate was present in the nasal cavity (Plates 1, 2). At this highest exposure concentration, squamous metaplasia of the larynx occurred in 1 male and 1 female rat. Microscopic lesions in rats exposed to 250 ppm were slightly less severe than in the 500 ppm group; inflammation and squamous metaplasia of the larynx were not present at this exposure concentration.

**TABLE 3 Histopathologic Lesions in F344/N Rats in the 2-Week Inhalation Studies of Formic Acid**

| | Exposure Concentration (ppm) | | | | | |
|---|---|---|---|---|---|---|
| Site/Lesion | 0 | 31 | 62.5 | 125 | 250 | 500 |
| **MALE** | | | | | | |
| **Nose** | | | | | | |
| **Respiratory epithelium** | | | | | | |
| squamous metaplasia | 0 | 0 | 4 (1.3)[a] | 5 (1.8) | 5 (2.8) | 5 (2.6) |
| inflammation | 0 | 0 | 0 | 3 (1.0) | 5 (2.4) | 5 (3.0) |
| necrosis | 0 | 0 | 0 | 0 | 5 (2.0) | 5 (2.6) |
| **Olfactory epithelium** | | | | | | |
| necrosis | 0 | 0 | 0 | 1 (1.0) | 2 (2.5) | 5 (2.6) |
| **Larynx** | | | | | | |
| squamous metaplasia | 0 | 0 | 0 | 0 | 0 | 1 (1.0) |
| inflammation | 0 | 0 | 0 | 0 | 0 | 2 (1.5) |
| **FEMALE** | | | | | | |
| **Nose** | | | | | | |
| **Respiratory epithelium** | | | | | | |
| squamous metaplasia | 0 | 0 | 3 (1.6) | 5 (2.6) | 5 (3.0) | 5 (3.0) |
| inflammation | 0 | 0 | 0 | 4 (1.3) | 5 (2.0) | 5 (3.0) |
| necrosis | 0 | 0 | 0 | 0 | 3 (1.6) | 5 (3.0) |
| **Olfactory epithelium** | | | | | | |
| necrosis | 0 | 0 | 0 | 1 (1.0) | 4 (1.5) | 5 (3.0) |
| **Larynx** | | | | | | |
| squamous metaplasia | 0 | 0 | 0 | 0 | 0 | 1 (1.0) |
| inflammation | 0 | 0 | 0 | 0 | 1 (1.0) | 1 (2.0) |

[a] Incidence and severity score ( ) based on a scale of 1 to 4: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked. Scores are averages based on the number of animal with lesions from groups of 5.

Rats exposed to 125 ppm formic acid had a decreased severity and incidence of nasal lesions when compared to those in the higher exposure groups; histopathologic lesions generally consisted of a minimal-to-mild squamous metaplasia of the respiratory epithelium on the nasal septum, lateral walls, and tips of the nasoturbinates (Plate 3). Minimal focal necrosis of the olfactory epithelium occurred in 2 rats at this exposure concentration. In the 62.5 ppm exposure groups, lesions were limited to the most anterior nasal section (Level I) and consisted of minimal-to-mild squamous metaplasia of the respiratory epithelium (Table 3). There were no microscopic lesions in the olfactory epithelium at this exposure concentration, and no treatment-related lesions in rats exposed to 31.5 ppm formic acid. No lesions in the lower respiratory tract were considered related to formic acid exposure at any concentration studied.

## 13-Week Studies in Rats

All rats survived to the end of the studies. Male rats exposed to 32 ppm formic acid had a mild but significant increase in final body weight compared to control animals (Table 4 and Figure 1). Similarly, body weight gains were significantly greater in male rats exposed to 16, 32, and 64 ppm formic acid compared to control animals (Table 4 and Figure 1). No clinical signs that were clearly exposure-related were noted during the studies.

**TABLE 4 Survival and Weight Gain of F344/N Rats in the 13-Week Inhalation Studies of Formic Acid**

| Exposure Concentration (ppm) | Survival[a] | Mean Body Weight (grams) | | | Final Weight Relative to Controls (%)[c] |
|---|---|---|---|---|---|
| | | Initial | Final | Change[b] | |
| **MALE** | | | | | |
| 0 | 10/10 | 119 | 339 | 220 | |
| 8 | 10/10 | 124 | 357 | 233 | 105 |
| 16 | 10/10 | 117 | 357 | 240 | 105 |
| 32 | 10/10 | 123 | 367 | 244 | 108 |
| 64 | 10/10 | 119 | 362 | 243 | 107 |
| 128 | 10/10 | 121 | 333 | 212 | 98 |
| **FEMALE** | | | | | |
| 0 | 10/10 | 113 | 212 | 99 | |
| 8 | 10/10 | 113 | 210 | 97 | 99 |
| 16 | 10/10 | 112 | 205 | 93 | 97 |
| 32 | 10/10 | 111 | 208 | 97 | 98 |
| 64 | 10/10 | 111 | 205 | 94 | 97 |
| 128 | 10/10 | 108 | 201 | 93 | 95 |

a Number surviving at 13 weeks/number of animals per dose group.
b Mean weight change of the animals in each dose group.
c (Dosed group mean/Control group mean) x 100.

Changes in hematologic variables were few and generally minimal to mild in magnitude. Increases in white blood cell (WBC) counts in male and female rats at 3 days were produced by mild lymphocytoses. RBC counts were significantly increased in male rats in the 2 highest exposure groups at day 3. Although there were no statistically significant changes in WBC counts at the 13-week time point, neutrophil counts were decreased in male and female rats in all exposure groups. The decreases were mild to moderate and not dependent on the exposure concentration. In the female rats at 23 days, mild but significant increases in MCH and MCV were produced by minimal

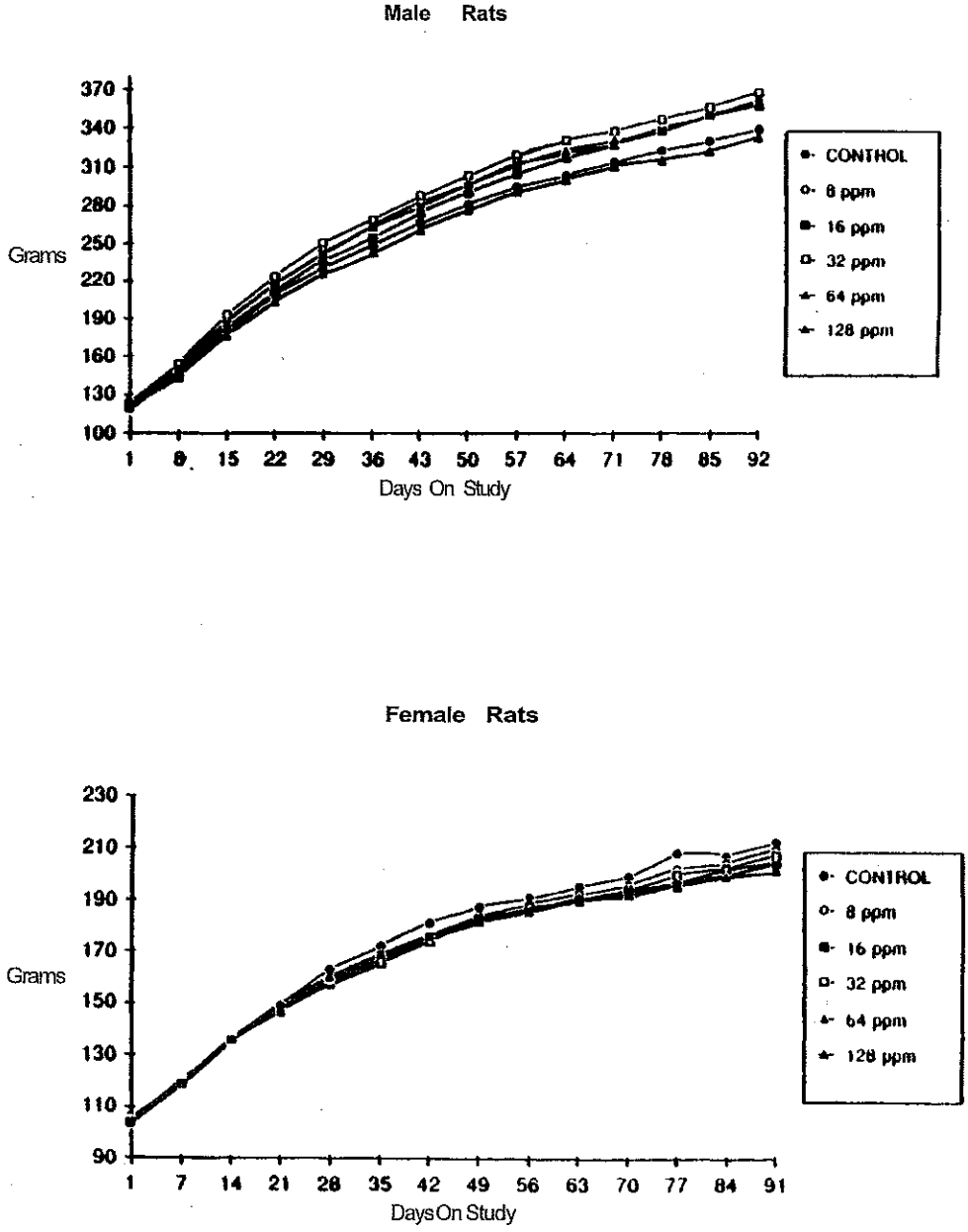


**Figure 1 Body Weights of F344/N Rats In the 13-Week Inhalation Studies of Formic Acid**

to mild decreases in RBC counts. In female rats at 13 weeks, there were minimal but significant increases in MCHC in animals at all exposure concentrations, produced by increases in HGB concentrations that were occasionally significant. Minimal but significant decreases in MCV in female rats in 2 exposure groups (16 and 128 ppm) at 13 weeks were associated with increases of similar magnitude in RBC counts.

There were mild, significant decreases in concentrations of serum albumin in female rats at day 3 (32, 64, and 128 ppm exposure groups) and increases in male rats at 13 weeks (8, 16, and 32 ppm exposure groups). Concentrations of total serum protein were decreased in female rats in all exposure groups at day 3. Male and female rats exposed to 16, 32 (female only), 64, and 128 ppm formic acid had significant increases in serum AP at 13 weeks. Additional changes in serum biochemical variables in rats exposed to formic acid included decreases in activities of amylase (female rats, days 3 and 23) and CK (male rats, day 3; female rats, day 23), increases in activities of SDH (male rats, day 3), and decreases in concentrations of UN and creatinine (male and female rats, day 3).

There were no unusual gross lesions noted at necropsy. Liver weights were somewhat greater in male rats in all exposure groups and liver-to-body-weight ratios (relative weights) were increased in male rats exposed to 32, 64, and 128 ppm formic acid (Appendix A). Absolute and relative lung weights were decreased in all exposed groups of female rats. In male rats, relative lung weights were decreased in all exposure groups, and absolute weights were decreased in the 64 and 128 ppm groups.

Microscopic changes attributed to formic acid exposure occurred in the respiratory and olfactory epithelium of the nose and generally were limited to the 128 ppm exposure groups (Table 5).

**TABLE 5 Histopathologic Lesions in F344/N Rats in the 13-Week Inhalation Studies of Formic Acid**

| | Exposure Concentration (ppm) | | | | | |
|---|---|---|---|---|---|---|
| Site/Lesion | 0 | 8 | 16 | 32 | 64 | 128 |
| **MALE** | | | | | | |
| **NOSE** | | | | | | |
| **Respiratory epithelium** | | | | | | |
| squamous metaplasia | 0 | 0 | 0 | 0 | 0 | 9 (1.0)[a] |
| **Olfactory epithelium** | | | | | | |
| degeneration | 0 | 0 | 0 | 1 (1.0) | 1 (1.0) | 9 (1.2) |
| **FEMALE** | | | | | | |
| **NOSE** | | | | | | |
| **Respiratory epithelium** | | | | | | |
| squamous metaplasia | 0 | 0 | 0 | 0 | 0 | 6 (1.4) |
| **Olfactory epithelium** | | | | | | |
| degeneration | 0 | 0 | 0 | 0 | 0 | 5 (1.0) |

a Incidence and severity score ( ) based on a scale of 1 to 4: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked. Scores are averages based on the number of animals with lesions from groups of 10.

Changes in the respiratory epithelium consisted of a minimal squamous metaplasia in which the pseudostratified, ciliated columnar cells were replaced by a flattened, non-ciliated epithelium varying from approximately 2 to 5 cells in thickness. A few inflammatory cells sometimes were associated with these areas of metaplasia, but inflammation was not a prominent feature of the nasal lesions. Squamous metaplasia occurred most often in the respiratory epithelium that lines the most dorsal portion of the dorsal meatus in the nose's anterior section (Level I). Foci of squamous metaplasia occasionally were present on the anterior nasal septum and/or tips (margins) of the nasoturbinates (Plate 4). In the olfactory epithelium, degenerative changes were minimal to mild and generally limited to the area of the dorsal meatus in the mid-nasal section (Level II). Degeneration was characterized by a loss of the usual orderly arrangement of the pseudostratified layer of nuclei and by a slight reduction in the normal thickness of the olfactory epithelium. This decreased thickness was the result of a reduction in the amount of the cytoplasm at the apical portion of the olfactory epithelial cells and a decrease in the number of sensory and sustentacular cell nuclei (Plates 5, 6). An increase in the basophilic staining of some nuclei was seen, and, in a few cells, the nucleus appeared pyknotic, or fragmented; however, necrosis was not a characteristic feature of the olfactory lesion. There was no evidence of metaplasia in the olfactory epithelium or atrophy of the nerve fibers in the olfactory mucosa.

In 19/20 male and female rats from the control and 32 ppm exposure groups there were minimal to mild inflammatory lesions in the lung consisting of aggregates of macrophages and/or neutrophils in alveoli and hyperplasia of peribronchiolar lymphoid tissues and alveolar epithelium. These pulmonary lesions, which were generally less severe in females, were limited to the control and mid-dose groups and corresponded to the slightly greater lung weights observed for these groups of rats.

There were no effects of exposure to formic acid on measures of sperm motility, density, or testicular or epididymal weights, and no changes were seen in the length of the estrous cycle (Appendix C).

# Plates

**Plate 1.** Nasoturbinate from male rat exposed to 500 ppm formic acid in the 2-week study shows prominent squamous metaplasia (M) of the respiratory mucosa, and inflammatory cell exudate (E) on the mucosal surface. 330X

**Plate 2.** Medial septum of male rat exposed to 500 ppm formic acid in the 2-week study shows squamous metaplasia (M) of the respiratory mucosa, with keratin and an inflammatory cell exudate (arrows) along the mucosal surface. 330X

**Plate 3.** Nasal turbinate of female rat exposed to 125 ppm formic acid in the 2-week study shows minimal squamous metaplasia of respiratory mucosa on tip of turbinate (arrows). Compare with Plate 4, showing similar nasal lesion at same dose after 13 weeks of exposure. 330 X

**Plate 4.** Nasal turbinate of female rat exposed to 128 ppm formic acid in the 13-week study shows minimal squamous metaplasia of the respiratory mucosa on the tip of turbinate (arrows). Note the similar severity to that seen in Plate 3 with the same dose in the 2-week study. 330X

**Plate 5.** Olfactory mucosa from dorsal meatus of female rat exposed to 128 ppm formic acid in the 13-week study shows degeneration of the olfactory epithelial layer. Note the thinning of the apical cytoplasm (arrows) and slight decreased thickness of the nuclear layer compared to the control in Plate 6. 330X

**Plate 6.** Olfactory mucosa from dorsal meatus of control female rat in the 13-week study, for comparison with minimal degeneration in Plate 5. 330X

**Plate 7.** Nasal turbinate from female mouse exposed to 125 ppm formic acid in the 2-week study shows minimal squamous metaplasia of respiratory epithelium on turbinate. Compare to normal turbinate from control in Plate 8. 330 X

**Plate 8.** Nasal turbinate from control female mouse in the 2-week study shows normal cuboidal to columnar, ciliated (arrows) respiratory epithelium. 330X

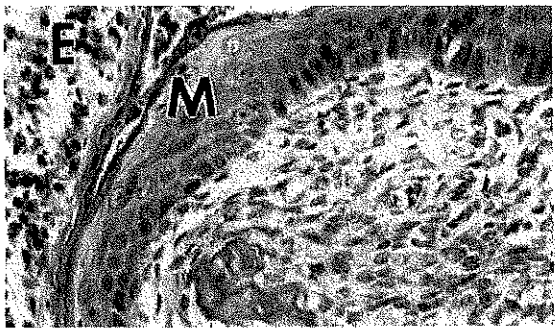


Plate 1

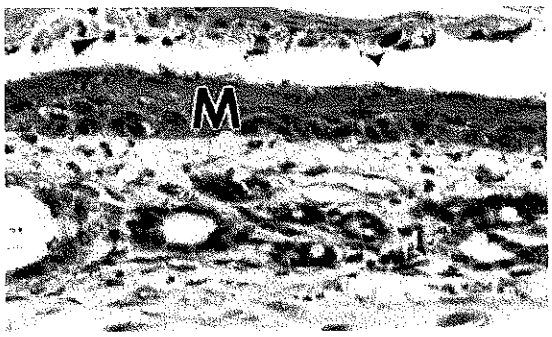


Plate 2

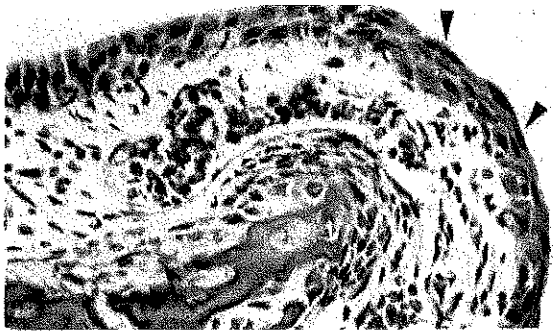

Plate 3

Plate 4

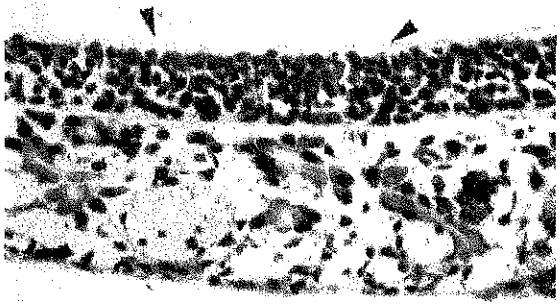

Plate 5

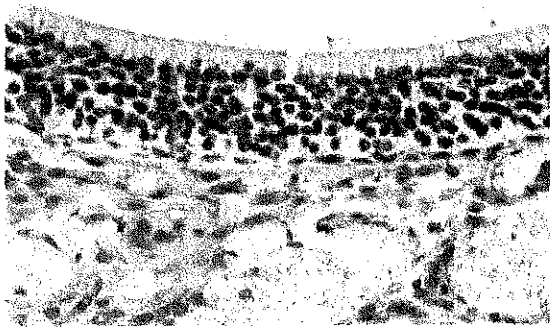

Plate 6

Plate 7

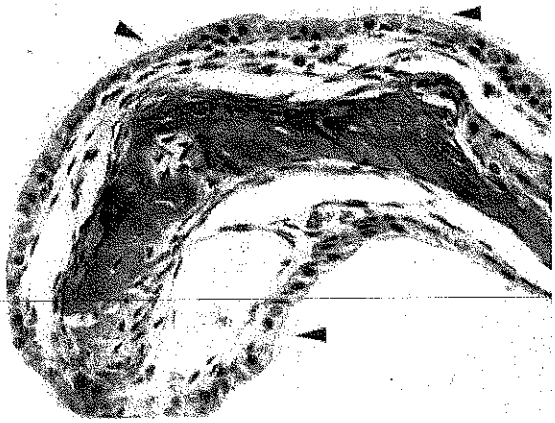

Plate 8

## 2-Week Studies in Mice

All mice exposed to 500 ppm of formic acid died during the first week of study; a female mouse from the 250 ppm exposure group became moribund and was killed on day 4. At the end of the study, body weight gain was significantly less than controls in the 250 ppm exposure groups (Table 6). Exposure-related clinical signs were limited to the 250 and 500 ppm exposure groups. These signs were consistent for effects produced by respiratory irritants and included nasal discharge, increased preening, and labored breathing. Corneal opacity was present in the highest dose groups of male and female mice.

**TABLE 6 Survival and Weight Gain of B6C3F$_1$ Mice in the 2-Week Inhalation Studies of Formic Acid**

| Exposure Concentration (ppm) | Survival[a] | Mean Body Weight (grams) Initial | Final | Change[b] | Final Weight Relative to Controls (%)[c] |
|---|---|---|---|---|---|
| **MALE** | | | | | |
| 0.0 | 5/5 | 22.8 | 25.8 | 3.0 | |
| 31.0 | 5/5 | 22.6 | 25.3 | 2.7 | 98 |
| 62.5 | 5/5 | 22.7 | 25.5 | 2.8 | 99 |
| 125.0 | 5/5 | 23.0 | 24.7 | 1.7 | 96 |
| 250.0 | 5/5 | 23.1 | 21.0 | -2.1 | 81 |
| 500.0 | 0/5 | 22.6 | - | - | - |
| **FEMALE** | | | | | |
| 0.0 | 5/5 | 19.6 | 22.5 | 2.9 | |
| 31.0 | 5/5 | 19.5 | 22.5 | 3.0 | 100 |
| 62.5 | 5/5 | 19.6 | 22.7 | 3.1 | 101 |
| 125.0 | 5/5 | 19.8 | 21.5 | 1.7 | 96 |
| 250.0 | 4/5 | 19.2 | 18.9 | -0.3 | 84 |
| 500.0 | 0/5 | 19.8 | - | - | - |

a Number surviving at 14 days/number of animals per dose group.
b Mean weight change of the animals in each dose group.
c (Dosed group mean/control group mean) x 100.

At necropsy, exposure-related gross lesions consisted of dried exudate around the external nares of all mice from the 500 ppm exposure groups and the 1 female from the 250 ppm exposure groups that died during the first week of the study. In mice that died during the study, segmental portions of the gastrointestinal tract (stomach and small intestine) were distended with air. Distention is attributed to the swelling and occlusion of nasal passages and subsequent swallowing of air which occurs when an obligate nose-breathing animal must breathe by mouth. There were no exposure-related gross lesions in mice necropsied at the end of the study. There were small increases (~10%) in the relative kidney weight in males exposed to 62.5, 125, and 250 ppm and in females exposed to 250 ppm. Thymus weights were reduced on an absolute and relative basis in mice exposed to 250 ppm; relative lung weights were increased mildly in these groups.

Histopathologic lesions were similar in male and female mice. They were limited to the nasal passages, except at the highest dose where they also were present in the larynx, pharynx, and trachea (Table 7). At 500 ppm, exposure-related lesions were of greatest severity in the most anterior section (Level I) of the nose and consisted of necrosis of the respiratory epithelium, with an accumulation of inflammatory cells in the mucosa and lumen of the nasal cavity. Squamous metaplasia of the respiratory epithelium generally was not present, but occasionally a basophilic-

**TABLE 7 Histopathologic Lesions in B6C3F$_1$ Mice in the 2-Week Inhalation Studies of Formic Acid**

| | Exposure Concentration (ppm) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 31 | 62.5 | 125 | 250 | 500 |
| **MALE** | | | | | | |
| **Nose** | | | | | | |
| **Respiratory epithelium** | | | | | | |
| squamous metaplasia | 0 | 0 | 0 | 3 (1.3)[a] | 4 (1.3) | 1 (1.0) |
| inflammation | 0 | 0 | 0 | 2 (1.0) | 4 (1.2) | 5 (1.4) |
| necrosis | 0 | 0 | 0 | 0 | 0 | 4 (3.5) |
| **Olfactory epithelium** | | | | | | |
| degeneration | 0 | 0 | 0 | 0 | 3 (1.3) | 1 (2.0) |
| necrosis | 0 | 0 | 0 | 0 | 0 | 3 (2.0) |
| **Larynx** | | | | | | |
| squamous metaplasia | 0 | 0 | 0 | 0 | 0 | 5 (2.8) |
| inflammation | 0 | 0 | 0 | 0 | 0 | 3 (1.0) |
| **Pharynx** | | | | | | |
| necrosis | 0 | 0 | 0 | 0 | 0 | 3 (2.0) |
| **FEMALE** | | | | | | |
| **Nose** | | | | | | |
| **Respiratory epithelium** | | | | | | |
| squamous metaplasia | 0 | 0 | 2 (1.0) | 3 (1.3) | 4 (1.0) | 0 |
| inflammation | 0 | 0 | 0 | 2 (1.5) | 5 (1.4) | 5 (1.8) |
| necrosis | 0 | 0 | 0 | 0 | 2 (1.5) | 5 (3.6) |
| **Olfactory epithelium** | | | | | | |
| degeneration | 0 | 0 | 0 | 0 | 2 (2.0) | 0 |
| necrosis | 0 | 0 | 0 | 0 | 1 (1.0) | 5 (1.8) |
| **Larynx** | | | | | | |
| squamous metaplasia | 0 | 0 | 0 | 0 | 0 | 1 (2.0) |
| inflammation | 0 | 0 | 0 | 0 | 0 | 3 (1.0) |
| necrosis | 0 | 0 | 0 | 0 | 0 | 5 (2.2) |
| **Pharynx** | | | | | | |
| necrosis | 0 | 0 | 0 | 0 | 0 | 2 (1.0) |

[a] Incidence and severity score ( ) based on a scale of 1 to 4: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked. Scores are averages based on the number of animals with lesions from groups of 5.

staining, flattened cuboidal cell partially covered the mucosal surface in areas with necrosis. Morphologic features of these cells were suggestive of regeneration; this was more prominent in the mice which survived longer.

Necrosis of respiratory and olfactory epithelium and nasal turbinate bone also was present in the midsection (Level II) of the nose. Focal areas of necrosis with associated inflammation and ulceration were present on the anterior portion of the pharyngeal hard palate. In female mice, there was necrosis, inflammation, and squamous metaplasia of the respiratory epithelium in the larynx. In the larynx of male mice, necrosis was not present, but squamous metaplasia was more prominent than in females. In the trachea of 2 of the 5 males from this exposure group, there was focal regeneration of the respiratory epithelium, morphologically similar to that seen in the nasal cavity. Mice exposed to 250 ppm of formic acid had a lower incidence and severity of necrosis of respiratory epithelium and nasal turbinate bone; in mice from this exposure concentration, necrosis of the olfactory epithelium was present in only a single female. There was a minimal to mild degeneration of the olfactory epithelium similar to that previously described for the rats. This degeneration was characterized by a decrease in thickness and loss of the normal arrangement of the pseudostratified nuclear layers comprising the olfactory epithelium. Squamous metaplasia of the respiratory epithelium was more prominent in the 250 ppm exposure groups when compared to

the 500 ppm groups which died during the first week of exposure. At an exposure concentration of 125 ppm of formic acid, squamous metaplasia and inflammation were present in 3 males and 3 females (Plates 7, 8); necrosis (minimal) of the respiratory epithelium was present in 1 female. There was no microscopic evidence for toxicity in the olfactory epithelium at this exposure concentration. There were no exposure-related changes in male mice exposed to concentrations below 125 ppm. At 62.5 ppm, there was minimal squamous metaplasia of the respiratory epithelium in 2 female mice; no microscopic evidence of toxicity was present in the 31 ppm exposure group of female mice.

## 13-Week Studies in Mice

There were no clinical signs or mortality associated with exposure of male or female mice to formic acid concentrations up to 128 ppm. Body weight gains were significantly less than controls in the 128 ppm exposure groups of both sexes and in female mice exposed to 64 ppm formic acid (Table 8, Figure 2). Changes in organ weights were limited largely to increases in relative weights in animals in the 128 ppm groups (Appendix A). This was primarily a reflection of the lower body weights of these animals compared to controls, and of the greater relative weight of organs in smaller animals. However, small increases in relative liver and kidney weights were seen in males and females, respectively, in the 32 and 64 ppm exposure groups.

**TABLE 8 Survival and Weight Gain of B6C3F$_1$ Mice in the 13-Week Inhalation Studies of Formic Acid**

| Exposure Concentration (ppm) | Survival[a] | Mean Body Weight (grams) | | | Final Weight Relative to Controls (%)[c] |
|---|---|---|---|---|---|
| | | Initial | Final | Change[b] | |
| **MALE** | | | | | |
| 0 | 10/10 | 26.4 | 35.3 | 8.9 | |
| 8 | 10/10 | 26.3 | 36.0 | 9.7 | 102 |
| 16 | 10/10 | 25.9 | 34.6 | 8.7 | 98 |
| 32 | 10/10 | 26.4 | 35.7 | 9.3 | 101 |
| 64 | 10/10 | 26.1 | 34.3 | 8.2 | 97 |
| 128 | 9/10 | 25.9 | 29.5 | 3.6 | 84 |
| **FEMALE** | | | | | |
| 0 | 10/10 | 20.8 | 31.9 | 11.1 | |
| 8 | 10/10 | 21.0 | 32.4 | 11.4 | 102 |
| 16 | 10/10 | 20.2 | 31.1 | 10.9 | 97 |
| 32 | 10/10 | 21.0 | 31.6 | 10.6 | 99 |
| 64 | 10/10 | 20.5 | 29.9 | 9.4 | 94 |
| 128 | 9/10 | 20.9 | 25.6 | 4.7 | 80 |

a Number surviving at 13 weeks/number of animals per dose group.
b Mean weight change of the animals in each dose group.
c (Dosed group mean/Control group mean) x 100.

There were no exposure-related gross lesions; microscopic changes attributed to toxicity of formic acid were limited to degeneration of the olfactory epithelium of the nose in a few mice from the 64 and 128 ppm exposure groups (Table 9). This minimal degeneration occurred in the dorsal portion of the dorsal meatus in the anterior or mid-nasal section (Levels I and II) and was similar to the olfactory degeneration described previously.

**TABLE 9 Histopathologic Lesions in B6C3F$_1$ Mice in the 13-Week Inhalation Studies of Formic Acid**

| | Exposure Concentration (ppm) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 8 | 16 | 32 | 64 | 128 |
| **MALE** | | | | | | |
| **Olfactory epithelium** degeneration | 0 | 0 | 0 | 0 | 0 | 2 (1.0)[a] |
| **FEMALE** | | | | | | |
| **Olfactory epithelium** degeneration | 0 | 0 | 0 | 0 | 2 (1.0) | 5 (1.0) |

[a] Incidence and severity score ( ) based on a scale of 1 to 4: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked. Scores are averages based on the number of animals with lesions from groups of 10.

There were no adverse effects of formic acid exposure on reproductive parameters evaluated in male or female mice (Appendix C). Sperm motility was somewhat lower in the exposed groups compared to controls, but the values for controls were rather high, and the values for exposed mice fall well within the historical range for control mice.

## Genetic Toxicity

Buffered solutions of formic acid were found not mutagenic in *Salmonella typhimurium* strains TA100, TA1535, TA97, and TA98, (Appendix D1).

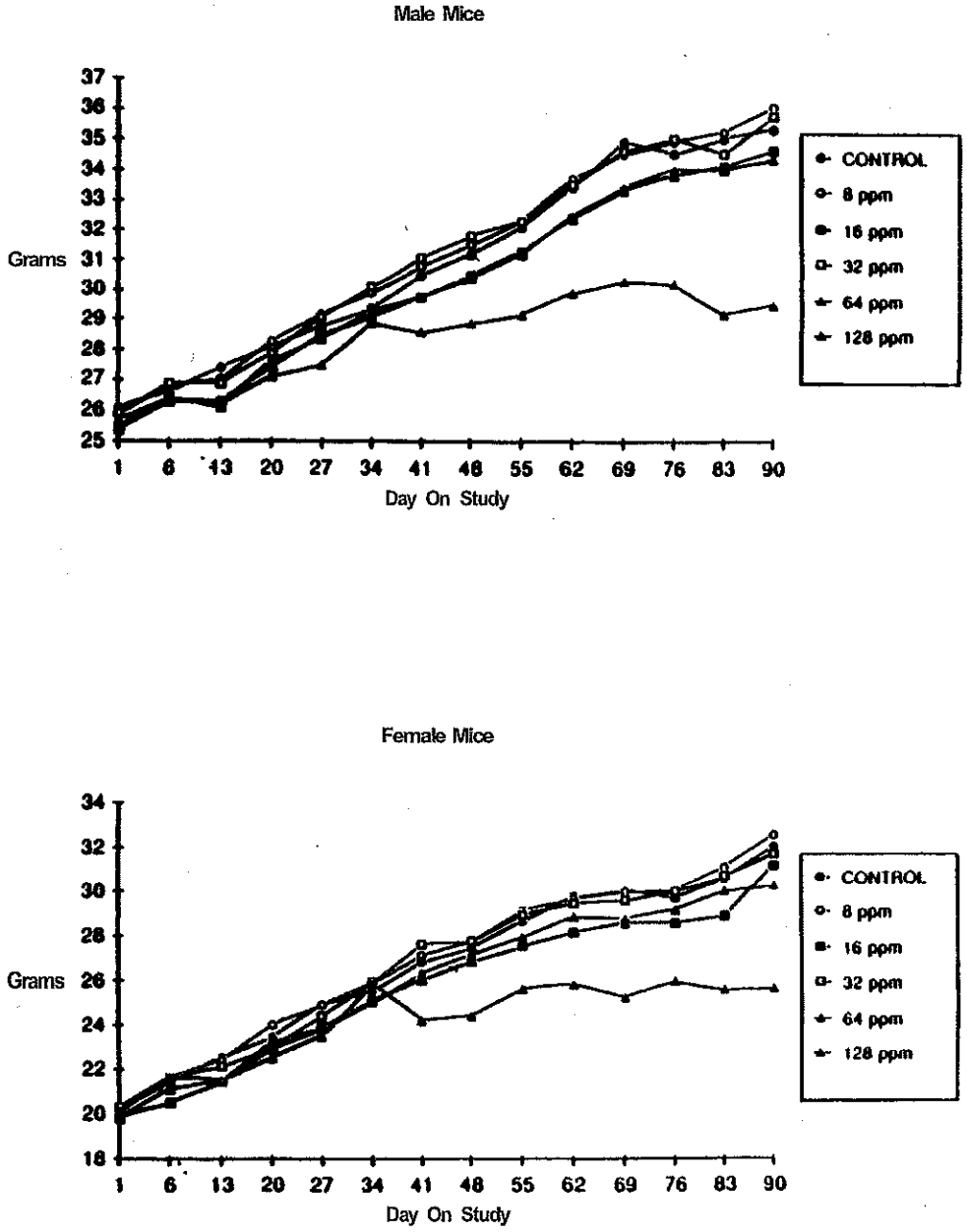


Figure 2 Body Weights of B6C3F$_1$ Mice In the 13-Week Inhalation Studies of Formic Acid

# DISCUSSION

The upper respiratory tract was the site for toxicity in rats and mice following exposure to formic acid by the inhalation route. In 2-week studies, toxicity, as evidenced primarily by necrosis and inflammation, was limited to the nasal passages, pharynx, larynx, and trachea of rats and mice exposed to concentrations of 250 and 500 ppm formic acid. Mice generally were more sensitive to the toxicity of formic acid at the highest exposure concentrations. Deaths were attributed to swelling of the nasal mucosa resulting in marked impairment of respiration. Because of mortality, clinical signs of labored breathing, and depression in body weight gain seen at exposure concentrations of 250 and 500 ppm in the 2-week studies, the highest exposure for the 13-week studies was limited to 128 ppm.

Microscopic lesions following exposure to 125 ppm formic acid for 2 weeks were limited to the nasal respiratory and olfactory epithelium in rats and the respiratory epithelium in mice. At the end of the 13-week studies there was little evidence for progression (in severity or incidence) of the respiratory or olfactory lesions at exposure concentrations equivalent to those in the 2-week studies. Exposure-related toxicity in the larynx, pharynx, or trachea that was seen in rats and mice at the 250 and 500 ppm exposure concentrations in the 2-week studies did not occur following 13 weeks of exposure to concentrations as high as 128 ppm.

Rats exposed to equivalent concentrations of formic acid for 2 weeks and 13 weeks had no increased severity of lesions in the nasal passages following the longer period of exposure. In fact, the minimal effects present after 2 weeks of exposure to 62.5 ppm formic acid were not evident in male or female rats exposed to 64 ppm for 13 weeks; and necrosis of the respiratory epithelium observed in rats after 2 weeks exposure to 125 ppm was not present in the 13-week study among rats in the 128 ppm exposure group. Squamous metaplasia of the respiratory epithelium was present after 13 weeks of exposure to 128 ppm formic acid, but the severity was equal to or less than that present at 2 weeks. After 13 weeks there was no evidence of hyperplasia, dysplasia, or development of a superficial layer of keratinized epithelium in the areas of squamous metaplasia. Adaptation of nasal respiratory epithelium to the irritant effects of formaldehyde vapor has been observed in the rat (Monticello, 1990). Six weeks of exposure to a vapor concentration of 6 ppm resulted in squamous metaplasia and hyperplasia of the respiratory epithelium. When the period of exposure was increased to 18 months, treatment-related microscopic lesions were no longer present.

Although olfactory epithelial necrosis was not present in the 13-week study, minimal degeneration of olfactory epithelium was present in most rats in the highest exposure groups. Absence of inflammation, hyperplasia, metaplasia, and nerve fiber atrophy in the olfactory mucosa is further indication of the minimal severity of the olfactory degeneration. There were no lesions in the lungs attributed to formic acid toxicity; the slightly greater absolute and relative lung weights which occurred in the control and the 32-ppm exposure groups were associated with minimal to mild inflammatory lesions of undetermined etiology. Morphologically identical inflammatory lesions with corresponding increased lung weights have been seen in control and treated rats from other toxicity studies (NTP, 1992); the occurrence of these lesions has not been related to chemical exposure in either incidence or severity.

In mice the squamous metaplasia of respiratory epithelium which developed after 14 days of exposure to formic acid at 125 ppm was not present after 13 weeks of exposure to 128 ppm. In contrast, however, the minimal olfactory degeneration in 7/20 mice exposed to 128 ppm for 13 weeks did not develop in mice exposed to a similar concentration (125 ppm) of formic acid during the shorter period of the 2-week study.

Hematologic changes in rats measured at 3 time points during the study were mild and generally unremarkable. Changes related to RBC variables could have been associated with minimal to mild hemoconcentration. Considering the changes in WBC variables, the lymphocytosis at day 3 of exposure is consistent with a physiologic response, as opposed to an immunologic response. Leukocytoses produced by endogenous release of epinephrine can result in increases in lymphocyte counts. However, these usually are accompanied by neutrophilia. An explanation for the neutropenia at 13 weeks (not dose-related) is not obvious. Causes of neutropenias include decreased production in the bone marrow, increased margination or sequestration in the peripheral circulation, and increased utilization by an inflammatory response. Based on histopathologic findings at 13 weeks, an inflammatory response does not appear to be an adequate explanation.

Changes in serum biochemical variables were few and mild. Decreases in concentrations of UN, albumin (and consequently, total protein), and creatinine at day 3 are consistent with a decreased intake of food. An increased concentration of albumin, as occurred in male rats at 13 weeks, is usually associated with hemoconcentration (mild). At 3 days, the increase in SDH activity in male rats exposed to formic acid indicates damage to hepatocytes. However, the lack of increase in other indicators of hepatocellular injury (ALT, bile acids) and the extent of the increase in activity of SDH suggest a minimal to mild effect. Increases in serum activities of ALP (13 weeks, male and female rats) generally are produced by disorders in bone, liver, or intestines. However, microscopic changes were not observed in these tissues, and the biologic importance of this finding, as well as of the decreases in activities of amylase (females) and CK (males), are not known.

The site-specific and morphological effects of formic acid on the upper respiratory tract in rats and mice are consistent with those produced by exposure to irritant chemicals administered by the inhalation route (Buckley *et al.,* 1984; Boorman *et al.,* 1987; Morgan *et al.,* 1990; Jiang *et al.,* 1983). A spectrum of histopathologic lesions in the upper respiratory tract after exposure to water-soluble irritant chemicals has been described (Morgan *et al.,* 1990; Buckley *et al.,* 1984). As seen in this study, the squamous epithelium lining the anterior portion of the nasal cavity typically is more resistant to the toxic effect of irritant gases, but lesions frequently occur at multiple locations in the anterior nasal cavity including the dorsal meatus, nasal septum, and the tips or margins of the nasoturbinates (Morgan *et al.,* 1990). Irritant chemicals administered at sufficiently high concentrations result not only in toxicity in the nasal cavity but at sites lower in the respiratory tract, including the pharynx, larynx, trachea, and lung (Boorman *et al., 1*987; Jiang *et al.,* 1983).

Aerosols containing particulates (dusts), including cobalt sulfate (Bucher *et al.,* 1990) and nickel compounds (Dunnick *et al.,* 1989), also have produced a similar spectrum of toxicity in the nasal cavity. Strong chemical irritants may produce marked necrosis, inflammation, or metaplasia at these sites as well as in the nasal cavity; inflammatory exudate or swelling of respiratory tract tissues may result in dyspnea or death. Necrosis of the turbinate bone seen in the 2-week studies

in mice is consistent with changes described for other chemicals that have caused extensive necrosis and ulceration of the respiratory mucosa on the nasal turbinates (Gross *et al.,* 1987; Monticello *et al.,* 1990). Similarly, irritant gases result in degenerative changes of the olfactory epithelium, most commonly in the most anterior portion of the dorsal meatus (Gaskell, 1990), the same site in the nose where olfactory degeneration occurred with exposure to formic acid. Despite the clinical signs (marked toxicity and mortality at the highest exposure concentrations of formic acid in the 2-week studies), at 125 ppm and below there were minimal histopathologic effects related to exposure. When compared with findings in the 2-week study, the incidence and severity of histopathologic changes in the respiratory epithelium of the nasal cavity from the 125 ppm exposure concentration groups suggest some adaptation to the irritant effects, following resolution of the initial injury. However, there appears to be less evidence of adaptation in the olfactory mucosa where, after 13 weeks, minimal degeneration occurred at a dose level (125 ppm) where no effect was seen in mice and only a minimal effect was seen in 2/10 rats following 2 weeks of exposure.

The lack of significant systemic effects from exposure of rats and mice to formic acid should be considered in light of the known differences in species susceptibilities to methanol toxicity. After ingestion or administration of methanol, human beings and primates can develop severe metabolic acidosis and blindness. Rodents are resistant to methanol toxicity and, consequently, extrapolation of results from methanol studies with non-primates to human beings is not possible (Tephley, 1991). In studies with monkeys, however, the administration of methanol produced clinical effects (depression, anorexia, weakness, vomiting, hyperpnea, tachypnea, and dilated unresponsive pupils), morphological effects (edema of optic disc and optic nerve), and biochemical findings (acidosis, decrease in concentrations of blood $HCO_3$) consistent with those described in cases of human poisoning (McMartin *et al.,* 1975; Hayreh *et al.,* 1977; Clay *et al.,* 1975; Baumbach *et al.,* 1977). Metabolic acidosis and ocular toxicity are produced by accumulation of formate, a metabolic intermediate in methanol catabolism (Clay *et al.,* 1975; Tephly, 1977). Ocular toxicity results from formate accumulation and is independent of the development of metabolic acidosis (Hayreh *et al.,* 1977). In primates and monkeys, the primary system for formate metabolism is the folate-dependent pathway which converts formate to $CO_2$ and tetrahydrofolate. Inhibition of this system by feeding folate-deficient diets to rats, or by exposure of rats and monkeys to nitrous oxide (which inhibits activity of methionine synthetase, the enzyme that catalyzes the conversion of 5-methyl tetrahydrofolate to tetrahydrofolate), decreases hepatic concentrations of tetrahydrofolate and decreases rates of formate oxidation (Eells *et al.,* 1981; 1982; 1983). Additionally, activity of 10-formyl tetrahydrofolate dehydrogenase, the enzyme that catalyzes the conversion of 10-formyl tetrahydrofolate (which results from the metabolism of formate and tetrahydrofolate) to $CO_2$ and tetrahydrofolate, is much lower in human and monkey liver than in rat liver (Johlin *et al.,* 1987). Therefore, the insensitivity of rodents to methanol and, consequently, formate toxicity results from high levels of hepatic tetrahydrofolate and rapid metabolism of 10-formyl tetrahydrofolate to $CO_2$ and tetrahydrofolate. In future studies of formic acid/formate toxicity, the use of more susceptible species such as nonhuman primates or swine should be considered.

In conclusion, buffered solutions of formic acid were not mutagenic in *Salmonella.* Inhalation exposures to formic acid for 2 and 13 weeks in F344/N rats and B6C3F$_1$ mice produced minimal systemic toxic effects. At 13 weeks, hematologic and biochemical changes were mild and consistent with hemoconcentration. Gross and microscopic changes were confined to the upper respiratory tract and were consistent with effects produced by irritant chemicals administered by

inhalation exposure. Effects on the respiratory and olfactory epithelium at 13 weeks consisted of squamous metaplasia (minimal, rats) and degeneration (minimal, rats and mice), respectively. Based on the findings in the 13-week studies, the no-observed-adverse-effect-level (NOAEL) for microscopic lesions in rats and mice was 64 ppm, but a lower NOAEL (32 ppm) was determined based on respiratory lesions present at the end of the 2-week study. The lack of systemic effects in either the 2- or 13-week studies may be related to the ability of rodents to rapidly metabolize formate to $CO_2$. Because humans metabolize formate less readily than rodents and are significantly more sensitive to its toxicity, caution should be used in considering the results of these studies in determining potential human risks associated with systemic exposure to formic acid.

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# APPENDIX A

# Organ Weights and Organ-Weight-to-Body-Weight Ratios

**TABLE A1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Inhalation Study of Formic Acid[1]**

| | 0 ppm | 8 ppm | 16 ppm | 32 ppm | 64 ppm | 128 ppm |
|---|---|---|---|---|---|---|
| **MALE** | | | | | | |
| n | 10 | 10 | 10 | 10 | 10 | 10 |
| Necropsy body wt | 339 ± 5 | 358 ± 10 | 358 ± 6 | 367 ± 6* | 365 ± 4* | 334 ± 6 |
| Heart | | | | | | |
| Absolute | 0 920 ± 0 021 | 0 984 ± 0 032 | 0 971 ± 0 014 | 1 021 ± 0 021** | 0 993 ± 0 019 | 0 966 ± 0 022 |
| Relative | 2 72 ± 0 04 | 2 75 ± 0 03 | 2 71 ± 0 02 | 2 78 ± 0 05 | 2 72 ± 0 04 | 2 90 ± 0 06** |
| Right Kidney | | | | | | |
| Absolute | 1 10 ± 0 03 | 1 14 ± 0 05 | 1 14 ± 0 03 | 1 23 ± 0 02* | 1 17 ± 0 03 | 1 15 ± 0 03 |
| Relative | 3 23 ± 0 04 | 3 17 ± 0 08 | 3 18 ± 0 06 | 3 34 ± 0 04 | 3 21 ± 0 06 | 3 44 ± 0 06* |
| Liver | | | | | | |
| Absolute | 10 57 ± 0 29 | 11 74 ± 0 49* | 11 59 ± 0 27* | 12 41 ± 0 33** | 12 46 ± 0 30** | 11 15 ± 0 44** |
| Relative | 31 2 ± 0 60 | 32 7 ± 0 64 | 32 4 ± 0 57 | 33 8 ± 0 39** | 34 1 ± 0 50** | 33 3 ± 0 96** |
| Lungs | | | | | | |
| Absolute | 1 97 ± 0 06 | 1 76 ± 0 06 | 1 74 ± 0 06 | 1 93 ± 0 06 | 1 66 ± 0 05** | 1 61 ± 0 04** |
| Relative | 5 81 ± 0 18 | 4 90 ± 0 11** | 4 87 ± 0 12** | 5 28 ± 0 19** | 4 54 ± 0 12** | 4 81 ± 0 05** |
| Right Testis | | | | | | |
| Absolute | 1 40 ± 0 03 | 1 45 ± 0 03 | 1 46 ± 0 02 | 1 47 ± 0 02 | 1 44 ± 0 03 | 1 41 ± 0 03 |
| Relative | 4 13 ± 0 06 | 4 05 ± 0 07 | 4 07 ± 0 07 | 4 00 ± 0 06 | 3 95 ± 0 08 | 4 23 ± 0 08 |
| Thymus | | | | | | |
| Absolute | 0 378 ± 0 010 | 0 353 ± 0 012 | 0 341 ± 0 010 | 0 400 ± 0 024 | 0 355 ± 0 010 | 0 325 ± 0 012* |
| Relative | 1 12 ± 0 03 | 0 99 ± 0 03 | 0 95 ± 0 03 | 1 09 ± 0 07 | 0 97 ± 0 03* | 0 98 ± 0 05* |
| **FEMALE** | | | | | | |
| n | 10 | 10 | 10 | 10 | 10 | 10 |
| Necropsy body wt | 212 ± 4 | 208 ± 6 | 205 ± 5 | 207 ± 6 | 206 ± 4 | 202 ± 6 |
| Heart | | | | | | |
| Absolute | 0 658 ± 0 015 | 0 658 ± 0 017 | 0 645 ± 0 013 | 0 648 ± 0 019 | 0 627 ± 0 014 | 0 657 ± 0 014 |
| Relative | 3 10 ± 0 03 | 3 16 ± 0 05 | 3 15 ± 0 06 | 3 14 ± 0 04 | 3 04 ± 0 04 | 3 26 ± 0 06 |
| Right Kidney | | | | | | |
| Absolute | 0 721 ± 0 013 | 0 697 ± 0 023 | 0 730 ± 0 010 | 0 730 ± 0 018 | 0 718 ± 0 020 | 0 729 ± 0 018 |
| Relative | 3 40 ± 0 04 | 3 35 ± 0 09 | 3 57 ± 0 08 | 3 54 ± 0 09 | 3 48 ± 0 08 | 3 62 ± 0 06 |
| Liver | | | | | | |
| Absolute | 6 28 ± 0 18 | 6 32 ± 0 27 | 6 07 ± 0 18 | 6 15 ± 0 20 | 6 29 ± 0 27 | 5 91 ± 0 19 |
| Relative | 29 6 ± 0 68 | 30 2 ± 0 64 | 29 6 ± 0 63 | 29 8 ± 0 60 | 30 4 ± 1 02 | 29 3 ± 0 47 |
| Lungs | | | | | | |
| Absolute | 1 47 ± 0 06 | 1 24 ± 0 05** | 1 20 ± 0 03** | 1 25 ± 0 04** | 1 17 ± 0 03** | 1 24 ± 0 04** |
| Relative | 6 97 ± 0 30 | 5 96 ± 0 21** | 5 85 ± 0 17** | 6 06 ± 0 14** | 5 68 ± 0 10** | 6 18 ± 0 23** |
| Thymus | | | | | | |
| Absolute | 0 289 ± 0 019 | 0 280 ± 0 019 | 0 258 ± 0 008 | 0 267 ± 0 013 | 0 267 ± 0 010 | 0 272 ± 0 014 |
| Relative | 1 36 ± 0 08 | 1 34 ± 0 08 | 1 26 ± 0 05 | 1 30 ± 0 07 | 1 29 ± 0 04 | 1 34 ± 0 05 |

[1] Organ weights are given in grams, organ-weight-to-body weight ratios are given as mg organ weight/g body weight (mean ± standard error)

* Statistically significantly different (P≤0 05) from the control group by Williams' test or Dunnett's test

** Statistically significantly different (P≤0 01) from the control group by Williams' test or Dunnett's test

## TABLE A2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 13-Week Inhalation Study of Formic Acid[1]

| | 0 ppm | 8 ppm | 16 ppm | 32 ppm | 64 ppm | 128 ppm |
|---|---|---|---|---|---|---|
| **MALE** | | | | | | |
| n | 10 | 10 | 10 | 10 | 10 | 9 |
| Necropsy body weight | 35 5 ± 0 5 | 36 1 ± 0 7 | 34 6 ± 0 5 | 35 8 ± 0 8 | 34 5 ± 0 4 | 29 4 ± 0 3** |
| Heart | | | | | | |
| Absolute | 0 152 ± 0 004 | 0 145 ± 0 003 | 0 150 ± 0 002 | 0 148 ± 0 002 | 0 146 ± 0 002 | 0 140 ± 0 003* |
| Relative | 4 28 ± 0 09 | 4 02 ± 0 08 | 4 34 ± 0 06 | 4 15 ± 0 07 | 4 23 ± 0 08 | 4 77 ± 0 10** |
| Right Kidney | | | | | | |
| Absolute | 0 299 ± 0 009 | 0 314 ± 0 007 | 0 316 ± 0 005 | 0 313 ± 0 006 | 0 299 ± 0 007 | 0 286 ± 0 006 |
| Relative | 8 41 ± 0 21 | 8 69 ± 0 13 | 9 13 ± 0 12 | 8 76 ± 0 14 | 8 66 ± 0 20 | 9 72 ± 0 15** |
| Liver | | | | | | |
| Absolute | 1 53 ± 0 03 | 1 58 ± 0 03 | 1 53 ± 0 03 | 1 66 ± 0 03** | 1 64 ± 0 01* | 1 44 ± 0 02 |
| Relative | 43 00 ± 0 24 | 43 8 ± 0 53 | 44 3 ± 0 63 | 46 5 ± 0 44** | 47 5 ± 0 43** | 49 1 ± 0 57** |
| Lungs | | | | | | |
| Absolute | 0 241 ± 0 003 | 0 233 ± 0 002 | 0 234 ± 0 004 | 0 232 ± 0 008 | 0 225 ± 0 005* | 0 208 ± 0 003** |
| Relative | 6 80 ± 0 14 | 6 47 ± 0 13 | 6 76 ± 0 10 | 6 47 ± 0 12 | 6 52 ± 0 15 | 7 08 ± 0 14 |
| Right Testis | | | | | | |
| Absolute | 0 122 ± 0 003 | 0 121 ± 0 002 | 0 120 ± 0 003 | 0 122 ± 0 003 | 0 122 ± 0 002 | 0 120 ± 0 003 |
| Relative | 3 44 ± 0 10 | 3 36 ± 0 06 | 3 46 ± 0 08 | 3 41 ± 0 07 | 3 52 ± 0 05 | 4 07 ± 0 10** |
| Thymus | | | | | | |
| Absolute | 0 035 ± 0 003 | 0 036 ± 0 003 | 0 036 ± 0 001 | 0 034 ± 0 004 | 0 039 ± 0 002 | 0 033 ± 0 001 |
| Relative | 0 98 ± 0 07 | 1 01 ± 0 07 | 1 03 ± 0 03 | 0 93 ± 0 11 | 1 13 ± 0 06 | 1 12 ± 0 05 |
| **FEMALE** | | | | | | |
| n | 10 | 10 | 10 | 10 | 10 | 10 |
| Necropsy body wt | 32 7 ± 0 7 | 33 0 ± 0 9 | 31 0 ± 0 6 | 31 9 ± 0 7 | 29 4 ± 0 7** | 25 3 ± 0 3** |
| Heart | | | | | | |
| Absolute | 0 134 ± 0 002 | 0 130 ± 0 002 | 0 131 ± 0 002 | 0 129 ± 0 002 | 0 124 ± 0 003 | 0 130 ± 0 003 |
| Relative | 4 12 ± 0 13 | 3 97 ± 0 13 | 4 24 ± 0 11 | 4 05 ± 0 06 | 4 24 ± 0 11 | 5 13 ± 0 10** |
| Right Kidney | | | | | | |
| Absolute | 0 200 ± 0 003 | 0 209 ± 0 004 | 0 204 ± 0 003 | 0 216 ± 0 003* | 0 200 ± 0 005 | 0 207 ± 0 004 |
| Relative | 6 14 ± 0 15 | 6 38 ± 0 24 | 6 61 ± 0 19 | 6 79 ± 0 14* | 6 82 ± 0 16** | 8 18 ± 0 12** |
| Liver | | | | | | |
| Absolute | 1 52 ± 0 03 | 1 53 ± 0 05 | 1 52 ± 0 03 | 1 55 ± 0 03 | 1 41 ± 0 03* | 1 31 ± 0 03** |
| Relative | 46 6 ± 0 58 | 46 4 ± 1 04 | 49 00 ± 0 66 | 48 7 ± 0 69 | 48 00 ± 0 39 | 51 6 ± 0 66** |
| Lungs | | | | | | |
| Absolute | 0 239 ± 0 005 | 0 225 ± 0 005 | 0 237 ± 0 005 | 0 240 ± 0 005 | 0 243 ± 0 013 | 0 241 ± 0 014 |
| Relative | 7 33 ± 0 16 | 6 84 ± 0 14 | 7 68 ± 0 24 | 7 54 ± 0 17 | 8 24 ± 0 26* | 9 50 ± 0 48** |
| Thymus | | | | | | |
| Absolute | 0 054 ± 0 002 | 0 055 ± 0 003 | 0 044 ± 0 002 | 0 054 ± 0 003 | 0 047 ± 0 002 | 0 042 ± 0 002** |
| Relative | 1 66 ± 0 07 | 1 66 ± 0 12 | 1 42 ± 0 05 | 1 68 ± 0 08 | 1 62 ± 0 08 | 1 66 ± 0 09 |

[1] Organ weights are given in grams, organ-weight-to-body weight ratios are given as mg organ weight/g body weight (mean ± standard error)

* Statistically significantly different (P≤0 05) from the control group by Williams' test or Dunnett's test

** Statistically significantly different (P≤0 01) from the control group by Williams' test or Dunnett's test

# APPENDIX B

# Hematology and Clinical Chemistry

**TABLE B1 Hematology Data for F344/N Rats in the 13-Week Inhalation Study of Formic Acid[1]**

| Analysis | 0 ppm | 8 ppm | 16 ppm | 32 ppm | 64 ppm | 128 ppm |
|---|---|---|---|---|---|---|
| **MALE** | | | | | | |
| n | 10 | 10 | 10 | 10 | 10 | 10 |
| Hematocrit (mL/dL) | | | | | | |
| Day 3 | 41.8 ± 0.2 | 42.0 ± 0.4 | 42.1 ± 0.4 | 42.8 ± 0.5 | 43.2 ± 0.4* | 42.8 ± 0.4 |
| Day 23 | 45.3 ± 0.3 | 45.3 ± 0.6 | 45.9 ± 0.3 | 45.7 ± 0.3 | 44.8 ± 0.2 | 45.2 ± 0.3 |
| Week 13 | 44.0 ± 0.3 | 44.1 ± 0.3 | 44.0 ± 0.3 | 43.9 ± 0.2 | 43.2 ± 0.3 | 44.1 ± 0.2 |
| Hemoglobin (g/dL) | | | | | | |
| Day 3 | 14.2 ± 0.1 | 14.1 ± 0.1 | 14.1 ± 0.1 | 14.5 ± 0.2 | 14.7 ± 0.1* | 14.6 ± 0.1* |
| Day 23 | 15.6 ± 0.1 | 15.7 ± 0.2 | 15.7 ± 0.1 | 15.7 ± 0.1 | 15.5 ± 0.1 | 15.4 ± 0.3 |
| Week 13 | 15.9 ± 0.2 | 16.0 ± 0.1 | 16.0 ± 0.1 | 15.9 ± 0.1 | 15.7 ± 0.1 | 16.0 ± 0.1 |
| Erythrocytes ($10^6$/μL) | | | | | | |
| Day 3 | 7.93 ± 0.07 | 7.89 ± 0.08 | 7.95 ± 0.11 | 8.16 ± 0.10 | 8.24 ± 0.08* | 8.22 ± 0.08* |
| Day 23 | 8.98 ± 0.09 | 8.87 ± 0.13 | 9.01 ± 0.07 | 8.95 ± 0.08 | 8.80 ± 0.06 | 8.84 ± 0.06 |
| Week 13 | 9.78 ± 0.05 | 9.89 ± 0.08 | 9.77 ± 0.07 | 9.77 ± 0.08 | 9.63 ± 0.08 | 9.85 ± 0.05 |
| Reticulocytes ($10^6$/μL) | | | | | | |
| Day 3 | 350.2 ± 24.4 | 394.3 ± 24.5 | 400.2 ± 29.5 | 318.7 ± 25.4 | 347.2 ± 20.7 | 389.7 ± 37.2 |
| Day 23 | 237.3 ± 18.3 | 218.3 ± 8.2 | 226.7 ± 22.2 | 250.4 ± 20.2 | 236.3 ± 26.7 | 236.2 ± 26.9 |
| Week 13 | 181.8 ± 14.0 | 174.7 ± 19.0 | 184.3 ± 15.2 | 228.3 ± 19.2 | 224.1 ± 21.2 | 196.7 ± 12.8 |
| Nucleated erythrocytes ($10^3$/μL) | | | | | | |
| Day 3 | 0.80 ± 0.25 | 1.00 ± 0.39 | 0.70 ± 0.34 | 0.70 ± 0.21 | 1.10 ± 0.18 | 0.80 ± 0.44 |
| Day 23 | 0.10 ± 0.10 | 0.50 ± 0.22 | 0.10 ± 0.10 | 0.10 ± 0.10 | 0.40 ± 0.22 | 0.10 ± 0.10 |
| Week 13 | 0.10 ± 0.10 | 0.30 ± 0.21 | 0.20 ± 0.13 | 0.30 ± 0.21 | 0.10 ± 0.10 | 0.00 ± 0.00 |
| Mean cell volume (fL) | | | | | | |
| Day 3 | 52.7 ± 0.3 | 53.1 ± 0.2 | 53.0 ± 0.3 | 52.5 ± 0.2 | 52.6 ± 0.2 | 52.1 ± 0.2 |
| Day 23 | 50.5 ± 0.3 | 51.0 ± 0.3 | 51.0 ± 0.2 | 50.9 ± 0.2 | 51.0 ± 0.2 | 51.1 ± 0.3 |
| Week 13 | 44.9 ± 0.2 | 44.6 ± 0.2 | 45.0 ± 0.0 | 45.0 ± 0.2 | 45.0 ± 0.3 | 44.8 ± 0.2 |
| Mean cell hemoglobin (pg) | | | | | | |
| Day 3 | 17.9 ± 0.1 | 17.9 ± 0.1 | 17.7 ± 0.1 | 17.8 ± 0.1 | 17.8 ± 0.1 | 17.7 ± 0.1 |
| Day 23 | 17.4 ± 0.1 | 17.7 ± 0.1 | 17.5 ± 0.1 | 17.5 ± 0.1 | 17.6 ± 0.1 | 17.3 ± 0.3 |
| Week 13 | 16.3 ± 0.1 | 16.2 ± 0.1 | 16.4 ± 0.1 | 16.3 ± 0.1 | 16.3 ± 0.1 | 16.3 ± 0.1 |
| Mean cell hemoglobin concentration (g/dL) | | | | | | |
| Day 3 | 33.9 ± 0.1 | 33.6 ± 0.1 | 33.5 ± 0.2 | 33.8 ± 0.1 | 34.0 ± 0.1 | 34.1 ± 0.1 |
| Day 23 | 34.4 ± 0.2 | 34.7 ± 0.2 | 34.3 ± 0.1 | 34.4 ± 0.1 | 34.6 ± 0.1 | 34.0 ± 0.5 |
| Week 13 | 36.1 ± 0.2 | 36.4 ± 0.1 | 36.4 ± 0.1 | 36.2 ± 0.2 | 36.3 ± 0.2 | 36.4 ± 0.1 |
| Platelets ($10^3$/μL) | | | | | | |
| Day 3 | 698.3 ± 30.7 | 804.2 ± 23.6* | 726.0 ± 17.5 | 672.8 ± 17.4 | 708.4 ± 21.3 | 742.1 ± 13.7 |
| Day 23 | 623.7 ± 12.9 | 622.4 ± 7.7 | 622.4 ± 12.6 | 604.7 ± 16.9 | 629.8 ± 7.6 | 611.1 ± 20.8 |
| Week 13 | 570.4 ± 21.4 | 545.8 ± 8.8 | 514.0 ± 18.6 | 554.6 ± 8.9 | 541.6 ± 5.9 | 505.7 ± 9.2** |
| Leukocytes ($10^3$/μL) | | | | | | |
| Day 3 | 7.37 ± 0.32 | 7.60 ± 0.34 | 7.46 ± 0.23 | 8.12 ± 0.33 | 9.02 ± 0.30** | 8.79 ± 0.50** |
| Day 23 | 5.60 ± 0.20 | 5.74 ± 0.17 | 6.18 ± 0.29 | 5.90 ± 0.24 | 6.03 ± 0.24 | 5.91 ± 0.23 |
| Week 13 | 6.99 ± 0.42 | 5.51 ± 0.18* | 5.67 ± 0.26* | 6.60 ± 0.19 | 5.97 ± 0.23 | 5.69 ± 0.24 |
| Segmented neutrophils ($10^3$/μL) | | | | | | |
| Day 3 | 0.41 ± 0.08 | 0.46 ± 0.07 | 0.44 ± 0.06 | 0.49 ± 0.09 | 0.54 ± 0.12 | 0.47 ± 0.10 |
| Day 23 | 0.61 ± 0.07 | 0.39 ± 0.05 | 0.63 ± 0.15 | 0.53 ± 0.06 | 0.67 ± 0.10 | 0.50 ± 0.05 |
| Week 13 | 1.51 ± 0.14 | 0.93 ± 0.07** | 0.81 ± 0.06** | 1.01 ± 0.09** | 0.93 ± 0.10** | 0.92 ± 0.11** |
| Lymphocytes ($10^3$/μL) | | | | | | |
| Day 3 | 6.80 ± 0.31 | 7.02 ± 0.33 | 6.76 ± 0.21 | 7.45 ± 0.37 | 8.15 ± 0.27** | 8.16 ± 0.47* |
| Day 23 | 4.91 ± 0.19 | 5.18 ± 0.17 | 5.37 ± 0.28 | 5.25 ± 0.23 | 5.26 ± 0.21 | 5.32 ± 0.24 |
| Week 13 | 5.11 ± 0.36 | 4.37 ± 0.13 | 4.68 ± 0.27 | 5.28 ± 0.22 | 4.79 ± 0.26 | 4.55 ± 0.29 |
| Monocytes ($10^3$/μL) | | | | | | |
| Day 3 | 0.15 ± 0.02 | 0.11 ± 0.03 | 0.23 ± 0.07 | 0.17 ± 0.03 | 0.30 ± 0.06 | 0.14 ± 0.03 |
| Day 23 | 0.08 ± 0.02 | 0.15 ± 0.03 | 0.16 ± 0.03 | 0.11 ± 0.03 | 0.09 ± 0.01 | 0.09 ± 0.02 |
| Week 13 | 0.31 ± 0.07 | 0.19 ± 0.04 | 0.14 ± 0.03 | 0.25 ± 0.03 | 0.19 ± 0.04 | 0.18 ± 0.03 |

**TABLE B1 Hematology Data for F344/N Rats in the 13-Week Inhalation Study of Formic Acid (continued)**

| Analysis | 0 ppm | 8 ppm | 16 ppm | 32 ppm | 64 ppm | 128 ppm |
|---|---|---|---|---|---|---|
| **MALE** (continued) | | | | | | |
| Eosinophils ($10^3/\mu L$) | | | | | | |
| Day 3 | 0 01 ± 0 01 | 0 02 ± 0 01 | 0 03 ± 0 01 | 0 02 ± 0 01 | 0 04 ± 0 02 | 0 02 ± 0 01 |
| Day 23 | 0 01 ± 0 01 | 0 03 ± 0 01 | 0 02 ± 0 01 | 0 02 ± 0 01 | 0 02 ± 0 01 | 0 01 ± 0 01 |
| Week 13 | 0 07 ± 0 02 | 0 03 ± 0 01 | 0 05 ± 0 02 | 0 07 ± 0 01 | 0 07 ± 0 02 | 0 05 ± 0 02 |
| **FEMALE** | | | | | | |
| n | 10 | 10 | 10 | 10 | 10 | 10 |
| Hematocnt (ml/dL) | | | | | | |
| Day 3 | 45 3 ± 0 4 | 44 8 ± 0 3 | 45 0 ± 0 5 | 45 1 ± 0 5 | 45 8 ± 0 9 | 45 4 ± 0 6 |
| Day 23 | 47 7 ± 0 4 | 46 9 ± 0 3 | 47 1 ± 0 4 | 46 7 ± 0 4 | 46 8 ± 0 4[2] | 46 9 ± 0 2 |
| Week 13 | 44 0 ± 0 2 | 45 2 ± 0 2* | 44 7 ± 0 3 | 44 9 ± 0 4 | 44 2 ± 0 5 | 44 8 ± 0 3 |
| Hemoglobin (g/dL) | | | | | | |
| Day 3 | 15 2 ± 0 1 | 15 0 ± 0 1 | 15 1 ± 0 2 | 15 1 ± 0 2 | 15 5 ± 0 3 | 15 4 ± 0 2 |
| Day 23 | 16 4 ± 0 2 | 16 1 ± 0 1 | 16 3 ± 0 1 | 16 1 ± 0 1 | 16 2 ± 0 2[2] | 16 2 ± 0 1 |
| Week 13 | 15 9 ± 0 1 | 16 5 ± 0 1** | 16 3 ± 0 1 | 16 3 ± 0 2 | 16 1 ± 0 2 | 16 4 ± 0 1* |
| Erythrocytes ($10^6/\mu L$) | | | | | | |
| Day 3 | 8 57 ± 0 10 | 8 45 ± 0 07 | 8 44 ± 0 11 | 8 48 ± 0 11 | 8 77 ± 0 19 | 8 70 ± 0 15 |
| Day 23 | 9 26 ± 0 10 | 8 96 ± 0 09 | 9 01 ± 0 09 | 8 89 ± 0 07* | 8 98 ± 0 08[2] | 8 97 ± 0 06 |
| Week 13 | 9 10 ± 0 04 | 9 37 ± 0 05* | 9 34 ± 0 06* | 9 34 ± 0 10 | 9 23 ± 0 10 | 9 39 ± 0 06* |
| Reticulocytes ($10^6/\mu L$) | | | | | | |
| Day 3 | 256 9 ± 23 3 | 277 0 ± 44 2 | 299 3 ± 32 0 | 290 9 ± 28 1 | 268 3 ± 26 6 | 254 3 ± 26 5 |
| Day 23 | 137 6 ± 9 1 | 162 5 ± 14 6 | 134 3 ± 12 0 | 143 9 ± 11 2 | 152 1 ± 20 1[2] | 155 7 ± 14 9 |
| Week 13 | 163 7 ± 14 2 | 139 2 ± 16 2 | 145 5 ± 20 4 | 151 4 ± 11 7 | 156 9 ± 19 7 | 151 9 ± 10 9 |
| Nucleated erythrocytes ($10^3/\mu L$) | | | | | | |
| Day 3 | 0 90 ± 0 41 | 0 50 ± 0 22 | 0 30 ± 0 15 | 0 30 ± 0 15 | 0 40 ± 0 22 | 0 40 ± 0 22 |
| Day 23 | 0 10 ± 0 10 | 0 20 ± 0 13 | 0 10 ± 0 10 | 0 00 ± 0 00 | 0 00 ± 0 00[2] | 0 10 ± 0 10 |
| Week 13 | 0 30 ± 0 15 | 0 10 ± 0 10 | 0 00 ± 0 00 | 0 00 ± 0 00* | 0 00 ± 0 00* | 0 00 ± 0 00** |
| Mean cell volume (fL) | | | | | | |
| Day 3 | 53 0 ± 0 2 | 53 1 ± 0 1 | 53 3 ± 0 3 | 53 2 ± 0 2 | 52 3 ± 0 2* | 52 2 ± 0 3* |
| Day 23 | 51 6 ± 0 3 | 52 4 ± 0 3* | 52 1 ± 0 3 | 52 6 ± 0 2** | 52 1 ± 0 2*[2] | 52 3 ± 0 3* |
| Week 13 | 48 3 ± 0 2 | 48 2 ± 0 1 | 47 7 ± 0 2* | 48 1 ± 0 1 | 48 1 ± 0 1 | 47 8 ± 0 1* |
| Mean cell hemoglobin (pg) | | | | | | |
| Day 3 | 17 7 ± 0 1 | 17 8 ± 0 1 | 17 9 ± 0 1 | 17 8 ± 0 1 | 17 6 ± 0 1 | 17 7 ± 0 1 |
| Day 23 | 17 7 ± 0 1 | 18 0 ± 0 1* | 18 1 ± 0 1** | 18 1 ± 0 1** | 18 0 ± 0 1**[2] | 18 0 ± 0 1** |
| Week 13 | 17 5 ± 0 1 | 17 6 ± 0 0 | 17 5 ± 0 0 | 17 4 ± 0 0 | 17 4 ± 0 1 | 17 4 ± 0 0 |
| Mean cell hemoglobin concentration (g/dL) | | | | | | |
| Day 3 | 33 4 ± 0 1 | 33 5 ± 0 1 | 33 6 ± 0 1 | 33 4 ± 0 1 | 33 8 ± 0 1 | 34 0 ± 0 1** |
| Day 23 | 34 4 ± 0 1 | 34 4 ± 0 2 | 34 5 ± 0 1 | 34 4 ± 0 1 | 34 5 ± 0 1[2] | 34 5 ± 0 1 |
| Week 13 | 36 1 ± 0 1 | 36 4 ± 0 1** | 36 5 ± 0 1** | 36 2 ± 0 1* | 36 4 ± 0 1* | 36 6 ± 0 1** |
| Platelets ($10^3/\mu L$) | | | | | | |
| Day 3 | 690 0 ± 36 0 | 692 1 ± 16 8 | 699 3 ± 30 7 | 682 7 ± 20 8 | 653 8 ± 17 1 | 605 9 ± 40 4 |
| Day 23 | 562 5 ± 12 1 | 587 6 ± 13 2 | 573 6 ± 15 6 | 552 7 ± 38 5 | 612 8 ± 11 7[2] | 582 6 ± 8 4 |
| Week 13 | 575 6 ± 16 6 | 541 6 ± 9 2 | 530 3 ± 6 8 | 556 1 ± 19 6 | 542 5 ± 9 7 | 535 2 ± 13 2 |
| Leukocytes ($10^3/\mu L$) | | | | | | |
| Day 3 | 8 03 ± 0 20 | 8 92 ± 0 58** | 8 67 ± 0 26* | 8 96 ± 0 40 | 9 83 ± 0 41** | 9 84 ± 0 17** |
| Day 23 | 5 85 ± 0 29 | 5 45 ± 0 32 | 5 86 ± 0 30 | 5 90 ± 0 51 | 5 91 ± 0 18[2] | 6 03 ± 0 14 |
| Week 13 | 5 57 ± 0 29 | 5 28 ± 0 26 | 4 78 ± 0 26 | 4 95 ± 0 26 | 4 96 ± 0 38 | 5 65 ± 0 50 |

**TABLE B1 Hematology Data for F344/N Rats in the 13-Week Inhalation Study of Formic Acid (continued)**

| Analysis | 0 ppm | 8 ppm | 16 ppm | 32 ppm | 64 ppm | 128 ppm |
|---|---|---|---|---|---|---|
| **FEMALE** (continued) | | | | | | |
| Segmented neutrophils ($10^3/\mu L$) | | | | | | |
| Day 3 | 0 62 ± 0 05 | 0 72 ± 0 13 | 0 64 ± 0 09 | 0 75 ± 0 11 | 0 69 ± 0 07 | 0 75 ± 0 09 |
| Day 23 | 0 66 ± 0 17 | 0 60 ± 0 09 | 0 57 ± 0 07 | 0 49 ± 0 05 | 0 53 ± 0 08[2] | 0 44 ± 0 06 |
| Week 13 | 1 50 ± 0 15 | 0 61 ± 0 08** | 0 56 ± 0 09** | 0 68 ± 0 05** | 0 56 ± 0 06** | 0 64 ± 0 09** |
| Lymphocytes ($10^3/\mu L$) | | | | | | |
| Day 3 | 7 24 ± 0 18 | 7 94 ± 0 53* | 7 88 ± 0 22* | 8 01 ± 0 39* | 8 96 ± 0 47** | 8 95 ± 0 23** |
| Day 23 | 5 04 ± 0 29 | 4 68 ± 0 32 | 5 16 ± 0 28 | 5 27 ± 0 47 | 5 24 ± 0 22[2] | 5 48 ± 0 16 |
| Week 13 | 3 80 ± 0 27 | 4 51 ± 0 28 | 3 97 ± 0 23 | 4 11 ± 0 28 | 4 24 ± 0 33 | 4 78 ± 0 42 |
| Monocytes ($10^3/\mu L$) | | | | | | |
| Day 3 | 0 14 ± 0 02 | 0 18 ± 0 05 | 0 12 ± 0 03 | 0 15 ± 0 03 | 0 12 ± 0 03 | 0 10 ± 0 03 |
| Day 23 | 0 10 ± 0 02 | 0 12 ± 0 03 | 0 08 ± 0 03 | 0 10 ± 0 03 | 0 09 ± 0 02[2] | 0 08 ± 0 02 |
| Week 13 | 0 23 ± 0 05 | 0 15 ± 0 03 | 0 16 ± 0 03 | 0 14 ± 0 03 | 0 12 ± 0 02 | 0 17 ± 0 03 |
| Eosinophils ($10^3/\mu L$) | | | | | | |
| Day 3 | 0 03 ± 0 02 | 0 08 ± 0 02 | 0 03 ± 0 02 | 0 05 ± 0 02 | 0 07 ± 0 03 | 0 05 ± 0 02 |
| Day 23 | 0 04 ± 0 02 | 0 04 ± 0 01 | 0 05 ± 0 02 | 0 05 ± 0 02 | 0 05 ± 0 02[2] | 0 03 ± 0 02 |
| Week 13 | 0 05 ± 0 02 | 0 02 ± 0 01 | 0 05 ± 0 02 | 0 03 ± 0 02 | 0 04 ± 0 01 | 0 07 ± 0 02 |

[1] Mean ± standard error
[2] n=9
* Statistically significantly different (P≤0 05) from the control group by Dunn's test or Shirley's test
** Statistically significantly different (P≤0 01) from the control group by Dunn's test or Shirley's test

**TABLE B2 Clinical Chemistry Data for F344/N Rats in the 13-Week Inhalation Study of Formic Acid[1]**

| Analysis | 0 ppm | 8 ppm | 16 ppm | 32 ppm | 64 ppm | 128 ppm |
|---|---|---|---|---|---|---|
| **MALE** | | | | | | |
| n | 10 | 10 | 10 | 10 | 10 | 10 |
| Urea nitrogen (mg/dL) | | | | | | |
| Day 3 | 31 3 ± 1 0 | 29 3 ± 0 5 | 28 1 ± 0 6** | 26 2 ± 0 8** | 23 8 ± 0 5** | 24 4 ± 0 8** |
| Day 23 | 24 2 ± 0 9 | 26 9 ± 0 6 | 26 6 ± 0 5 | 25 5 ± 1 0 | 25 6 ± 0 7 | 23 9 ± 0 6 |
| Week 13 | 21 7 ± 0 8 | 22 4 ± 0 7 | 24 5 ± 1 3 | 23 1 ± 1 0 | 24 0 ± 1 0 | 24 5 ± 1 1 |
| Creatinine (mg/dL) | | | | | | |
| Day 3 | 0 83 ± 0 04 | 0 81 ± 0 06 | 0 76 ± 0 03 | 0 68 ± 0 04* | 0 62 ± 0 04** | 0 60 ± 0 04** |
| Day 23 | 0 76 ± 0 05 | 0 82 ± 0 04 | 0 76 ± 0 04 | 0 79 ± 0 05 | 0 82 ± 0 03 | 0 71 ± 0 03 |
| Week 13 | 0 79 ± 0 04 | 0 78 ± 0 01 | 0 82 ± 0 04 | 0 80 ± 0 03 | 0 78 ± 0 03 | 0 77 ± 0 03 |
| Total protein (g/dL) | | | | | | |
| Day 3 | 7 6 ± 0 2 | 7 3 ± 0 1 | 7 2 ± 0 1 | 7 2 ± 0 1 | 7 2 ± 0 1 | 7 0 ± 0 2* |
| Day 23 | 7 8 ± 0 1 | 8 0 ± 0 1 | 8 0 ± 0 1 | 7 8 ± 0 1 | 7 8 ± 0 1 | 7 7 ± 0 1 |
| Week 13 | 7 4 ± 0 1 | 7 4 ± 0 1 | 7 5 ± 0 1 | 7 6 ± 0 1 | 7 3 ± 0 1 | 7 2 ± 0 1* |
| Albumin (g/dL) | | | | | | |
| Day 3 | 5 4 ± 0 1 | 5 2 ± 0 1 | 5 1 ± 0 1 | 5 1 ± 0 1 | 5 1 ± 0 1 | 5 0 ± 0 1 |
| Day 23 | 5 2 ± 0 1 | 5 4 ± 0 0 | 5 3 ± 0 0 | 5 2 ± 0 1 | 5 2 ± 0 1 | 5 2 ± 0 1 |
| Week 13 | 4 6 ± 0 1 | 4 8 ± 0 1* | 4 9 ± 0 1** | 4 9 ± 0 1* | 4 8 ± 0 1 | 4 7 ± 0 1 |
| Globulin (g/dL) | | | | | | |
| Day 3 | 2 2 ± 0 1 | 2 1 ± 0 1 | 2 1 ± 0 1* | 2 2 ± 0 1 | 2 1 ± 0 1* | 2 0 ± 0 1* |
| Day 23 | 2 5 ± 0 1 | 2 6 ± 0 1 | 2 7 ± 0 1 | 2 6 ± 0 1 | 2 6 ± 0 0 | 2 6 ± 0 1 |
| Week 13 | 2 8 ± 0 1 | 2 6 ± 0 1** | 2 6 ± 0 0** | 2 7 ± 0 0* | 2 6 ± 0 0** | 2 5 ± 0 1** |
| A/G ratio | | | | | | |
| Day 3 | 2 4 ± 0 1 | 2 5 ± 0 1 | 2 5 ± 0 1 | 2 4 ± 0 1 | 2 5 ± 0 1 | 2 5 ± 0 1 |
| Day 23 | 2 1 ± 0 0 | 2 0 ± 0 0 | 2 0 ± 0 1 | 2 0 ± 0 1 | 2 0 ± 0 0 | 2 0 ± 0 0 |
| Week 13 | 1 6 ± 0 1 | 1 9 ± 0 1** | 1 9 ± 0 1** | 1 8 ± 0 1** | 1 9 ± 0 0** | 1 9 ± 0 1** |
| Alkaline phosphatase (IU/L) | | | | | | |
| Day 3 | 1,049 ± 27 | 1,059 ± 33 | 1,011 ± 33 | 1,042 ± 27 | 970 ± 13* | 921 ± 23** |
| Day 23 | 721 ± 16 | 727 ± 9 | 730 ± 17 | 763 ± 16 | 732 ± 19 | 689 ± 13 |
| Week 13 | 325 ± 14 | 334 ± 11 | 356 ± 7* | 354 ± 11 | 376 ± 7** | 375 ± 6** |
| Alanine aminotransferase (IU/L) | | | | | | |
| Day 3 | 43 ± 2 | 42 ± 1 | 44 ± 2 | 45 ± 1 | 41 ± 1 | 41 ± 2 |
| Day 23 | 34 ± 1 | 36 ± 2 | 38 ± 1 | 36 ± 1 | 33 ± 1 | 32 ± 1 |
| Week 13 | 62 ± 5 | 66 ± 4 | 69 ± 10 | 62 ± 4 | 59 ± 3 | 56 ± 5 |
| Amylase (IU/L) | | | | | | |
| Day 3 | 5,529 ± 148 | 5,537 ± 117 | 5,606 ± 143 | 5,419 ± 78 | 5,443 ± 59 | 5,256 ± 119 |
| Day 23 | 6,223 ± 58 | 6,218 ± 169 | 6,272 ± 113 | 6,164 ± 130 | 6,366 ± 64 | 6,027 ± 96 |
| Week 13 | 6,431 ± 191 | 6,782 ± 122 | 6,775 ± 113 | 6,854 ± 119 | 6,620 ± 101 | 6,391 ± 93 |
| Creatine kinase (IU/L) | | | | | | |
| Day 3 | 752 ± 49 | 634 ± 34 | 508 ± 38** | 394 ± 19** | 412 ± 42** | 366 ± 54** |
| Day 23 | 207 ± 23 | 256 ± 20 | 245 ± 34 | 253 ± 29 | 218 ± 29 | 247 ± 30 |
| Week 13 | 131 ± 13 | 127 ± 19 | 151 ± 18 | 162 ± 19 | 140 ± 20 | 167 ± 24 |
| Sorbitol dehydrogenase (IU/L) | | | | | | |
| Day 3 | 6 ± 0 | 7 ± 1 | 9 ± 1** | 11 ± 1** | 9 ± 1** | 10 ± 1** |
| Day 23 | 10 ± 0 | 11 ± 0 | 10 ± 1 | 11 ± 0 | 10 ± 0 | 10 ± 1 |
| Week 13 | 15 ± 1 | 16 ± 1 | 18 ± 5 | 15 ± 1 | 15 ± 1 | 15 ± 2 |
| Bile acids (µmol/L) | | | | | | |
| Day 3 | 4 90 ± 1 05 | 6 35 ± 0 45 | 6 55 ± 1 38 | 5 75 ± 1 11 | 7 05 ± 1 69 | 5 75 ± 1 34 |
| Day 23 | 7 35 ± 0 71 | 7 20 ± 0 92 | 5 65 ± 0 73 | 6 10 ± 1 09 | 5 10 ± 0 82 | 10 88 ± 4 33 |
| Week 13 | 6 48 ± 1 70 | 4 45 ± 1 03 | 8 10 ± 2 53 | 4 23 ± 0 50 | 5 60 ± 0 74 | 7 42 ± 1 63 |

**TABLE B2 Clinical Chemistry Data for F344/N Rats in the 13-Week Inhalation Study of Formic Acid (continued)**

| Analysis | 0 ppm | 8 ppm | 16 ppm | 32 ppm | 64 ppm | 128 ppm |
|---|---|---|---|---|---|---|
| **FEMALE** | | | | | | |
| n | 10 | 10 | 10 | 10 | 10 | 10 |
| Urea nitrogen (mg/dL) | | | | | | |
| Day 3 | 33 4 ± 0 9 | 32 4 ± 0 9 | 30 8 ± 1 6* | 29 8 ± 0 8* | 28 9 ± 1 1** | 26 7 ± 1 0** |
| Day 23 | 28 3 ± 1 2 | 27 9 ± 1 2 | 27 6 ± 1 2 | 30 8 ± 1 1 | 27 3 ± 0 7[2] | 28 4 ± 1 1 |
| Week 13 | 25 4 ± 1 1 | 23 9 ± 1 1 | 24 6 ± 0 8 | 24 3 ± 1 1 | 23 8 ± 1 1 | 24 0 ± 1 1 |
| Creatinine (mg/dL) | | | | | | |
| Day 3 | 0 85 ± 0 04 | 0 76 ± 0 03 | 0 74 ± 0 03 | 0 73 ± 0 03* | 0 66 ± 0 02** | 0 63 ± 0 04** |
| Day 23 | 0 82 ± 0 06 | 0 84 ± 0 04 | 0 82 ± 0 05 | 1 00 ± 0 06 | 0 81 ± 0 05[2] | 0 83 ± 0 06 |
| Week 13 | 0 82 ± 0 02 | 0 71 ± 0 02** | 0 74 ± 0 02 | 0 78 ± 0 02 | 0 77 ± 0 03 | 0 73 ± 0 02* |
| Total protein (g/dL) | | | | | | |
| Day 3 | 7 5 ± 0 1 | 7 2 ± 0 1* | 7 1 ± 0 1* | 7 0 ± 0 1** | 6 8 ± 0 1** | 6 7 ± 0 1** |
| Day 23 | 7 8 ± 0 1 | 7 8 ± 0 1 | 7 7 ± 0 1 | 7 8 ± 0 1 | 7 7 ± 0 1[2] | 7 7 ± 0 1 |
| Week 13 | 7 4 ± 0 1 | 7 5 ± 0 1 | 7 5 ± 0 2 | 7 5 ± 0 1 | 7 4 ± 0 2 | 7 5 ± 0 1 |
| Albumin (g/dL) | | | | | | |
| Day 3 | 5 2 ± 0 1 | 5 0 ± 0 1 | 5 0 ± 0 1 | 4 9 ± 0 1** | 4 8 ± 0 1** | 4 7 ± 0 0** |
| Day 23 | 5 7 ± 0 1 | 5 6 ± 0 1 | 5 6 ± 0 1 | 5 6 ± 0 1 | 5 5 ± 0 1[2] | 5 7 ± 0 1 |
| Week 13 | 5 0 ± 0 1 | 5 2 ± 0 1 | 5 2 ± 0 1 | 5 1 ± 0 1 | 5 1 ± 0 1 | 5 2 ± 0 1 |
| Globulin (g/dL) | | | | | | |
| Day 3 | 2 3 ± 0 1 | 2 2 ± 0 1 | 2 1 ± 0 1 | 2 2 ± 0 1 | 2 0 ± 0 0** | 2 0 ± 0 1** |
| Day 23 | 2 1 ± 0 1 | 2 2 ± 0 0 | 2 0 ± 0 1 | 2 2 ± 0 1 | 2 2 ± 0 1[2] | 2 1 ± 0 1 |
| Week 13 | 2 4 ± 0 1 | 2 4 ± 0 1 | 2 4 ± 0 1 | 2 4 ± 0 1 | 2 4 ± 0 1 | 2 3 ± 0 0 |
| A/G ratio | | | | | | |
| Day 3 | 2 3 ± 0 1 | 2 3 ± 0 1 | 2 4 ± 0 1 | 2 3 ± 0 1 | 2 4 ± 0 0 | 2 4 ± 0 1 |
| Day 23 | 2 8 ± 0 1 | 2 6 ± 0 1 | 2 8 ± 0 1 | 2 6 ± 0 1 | 2 6 ± 0 1[2] | 2 8 ± 0 1 |
| Week 13 | 2 1 ± 0 1 | 2 2 ± 0 1 | 2 2 ± 0 1 | 2 2 ± 0 1 | 2 2 ± 0 1 | 2 2 ± 0 1 |
| Alkaline phosphatase (IU/L) | | | | | | |
| Day 3 | 995 ± 21 | 954 ± 28 | 989 ± 39 | 931 ± 33 | 864 ± 33** | 837 ± 47** |
| Day 23 | 702 ± 23 | 624 ± 21 | 655 ± 22 | 658 ± 20 | 629 ± 21[2] | 662 ± 27 |
| Week 13 | 351 ± 9 | 375 ± 9 | 400 ± 11** | 385 ± 9** | 392 ± 14* | 404 ± 13** |
| Alanine aminotransferase (IU/L) | | | | | | |
| Day 3 | 38 ± 2 | 37 ± 2 | 39 ± 2 | 39 ± 2 | 38 ± 2 | 37 ± 2 |
| Day 23 | 35 ± 2 | 31 ± 1 | 30 ± 1 | 32 ± 1 | 33 ± 2[2] | 33 ± 1 |
| Week 13 | 70 ± 13 | 51 ± 3 | 49 ± 3 | 52 ± 4 | 56 ± 6 | 57 ± 4 |
| Amylase (IU/L) | | | | | | |
| Day 3 | 4,230 ± 77 | 4,152 ± 58 | 4,125 ± 53 | 4,129 ± 57 | 3,873 ± 66** | 3,983 ± 95** |
| Day 23 | 4,587 ± 55 | 4,290 ± 126* | 4 412 ± 70 | 4,354 ± 61* | 4,206 ± 59**[2] | 4,206 ± 61** |
| Week 13 | 4,942 ± 200 | 4,973 ± 203 | 5,057 ± 112 | 5,076 ± 185 | 5,068 ± 405 | 4,704 ± 145 |
| Creatine kinase (IU/L) | | | | | | |
| Day 3 | 398 ± 41 | 373 ± 34 | 285 ± 31 | 270 ± 31* | 454 ± 75 | 305 ± 17 |
| Day 23 | 168 ± 16 | 231 ± 29 | 227 ± 19* | 233 ± 26* | 188 ± 24[2] | 251 ± 27* |
| Week 13 | 192 ± 32 | 130 ± 23 | 159 ± 25 | 211 ± 33 | 137 ± 20 | 122 ± 19 |
| Sorbitol dehydrogenase (IU/L) | | | | | | |
| Day 23 | 11 ± 1 | 10 ± 1 | 11 ± 0 | 11 ± 1 | 11 ± 1 | 11 ± 1 |
| Week 13 | 16 ± 3 | 14 ± 1 | 15 ± 1 | 14 ± 1 | 16 ± 1[2] | 14 ± 1 |
| Bile acids (µmol/L) | | | | | | |
| Day 3 | 6 90 ± 1 56 | 4 65 ± 0 73 | 7 25 ± 1 18 | 5 05 ± 0 31 | 8 15 ± 2 47 | 5 58 ± 0 65 |
| Day 23 | 7 85 ± 1 28 | 5 95 ± 0 96 | 7 35 ± 1 48 | 9 55 ± 1 54 | 7 83 ± 1 09[2] | 6 60 ± 1 05 |
| Week 13 | 12 00 ± 3 27 | 9 10 ± 1 09 | 8 45 ± 1 64 | 7 70 ± 0 72 | 9 20 ± 2 28 | 12 80 ± 3 43 |

[1] Mean ± standard error
[2] n=9
* Statistically significantly different (P≤0 05) from the control group by Dunn's test or Shirley's test
** Statistically significantly different (P≤0 01) from the control group by Dunn's test or Shirley's test

# APPENDIX C

# Reproductive Tissue Evaluations and Estrous Cycle Characterization

## TABLE C1 Summary of Reproductive Tissue Evaluations in Male F344/N Rats in the 13-Week Inhalation Study of Formic Acid

| Study Parameters[1] | 0 ppm | 8 ppm | 32 ppm | 128 ppm |
|---|---|---|---|---|
| **Weights (g)** | | | | |
| Necropsy body weight | 339 ± 5 | 358 ± 10 | 367 ± 6* | 334 ± 6 |
| Left epididymis | 0 449 ± 0 011 | 0 461 ± 0 011 | 0 469 ± 0 011 | 0 460 ± 0 004 |
| Left epididymal tail | 0 167 ± 0 007 | 0 171 ± 0 005 | 0 174 ± 0 007 | 0 169 ± 0 004 |
| **Spermatozoal measurements** | | | | |
| Motility (%) | 91 ± 1 | 91 ± 1 | 91 ± 1 | 88 ± 1 |
| Concentration ($10^6$/g) | 658 ± 21 | 706 ± 21 | 580 ± 60 | 651 ± 29 |

[1] Data presented as mean ± standard error, n=10 Differences from the control group for reproductive tissue weights and spermatozoal measurements are not significant by Dunn's test or Shirley's test

* Statistically significantly different (P≤0 05) from the control group by Dunnett's test

## TABLE C2 Summary of Estrous Cycle Characterization in Female F344/N Rats in the 13-Week Inhalation Study of Formic Acid

| Study Parameters[1] | 0 ppm | 8 ppm | 32 ppm | 128 ppm |
|---|---|---|---|---|
| **Necropsy body weight (g)** | 212 ± 4 | 208 ± 6 | 207 ± 6 | 202 ± 6 |
| **Estrous cycle length (days)** | 4 80 ± 0 15 | 4 75 ± 0 11 | 4 95 ± 0 05 | 4 95 ± 0 12 |
| **Estrous stages as % of cycle** | | | | |
| Diestrus | 38 3 | 39 2 | 41 7 | 40 8 |
| Proestrus | 17 5 | 18 3 | 19 2 | 19 2 |
| Estrus | 25 0 | 21 7 | 20 0 | 22 5 |
| Metestrus | 19 2 | 20 8 | 19 2 | 17 5 |

[1] Data presented as mean ± standard error, n=10 Necropsy body weights are not significant by Dunnett's test Estrous cycle lengths are not significant by Dunn's test or Shirley's test By multivariate analysis of variance (MANOVA), dosed groups do not differ significantly from controls in the relative length of time spent in estrous stages

## TABLE C3 Summary of Reproductive Tissue Evaluation in Male B6C3F, Mice in the 13-Week Inhalation Study of Formic Acid

| Study Parameters[1] | 0 ppm | 8 ppm | 32 ppm | 128 ppm[2] |
|---|---|---|---|---|
| **Weights (g)** | | | | |
| Necropsy body weight | 35 5 ± 0 5 | 36 1 ± 0 7 | 35 8 ± 0 8 | 29 4 ± 0 3** |
| Left epididymis | 0 042 ± 0 001 | 0 045 ± 0 001 | 0 042 ± 0 001 | 0 041 ± 0 001 |
| Left epididymal tail | 0 017 ± 0 001 | 0 017 ± 0 001 | 0 015 ± 0 000 | 0 015 ± 0 000* |
| **Spermatozoal measurements** | | | | |
| Motility (%) | 94 ± 0 | 91 ± 1** | 86 ± 1** | 90 ± 1** |
| Concentration ($10^6$/g) | 1060 ± 62 | 1114 ± 60 | 1337 ± 44** | 1406 ± 56** |

[1] Data presented as mean ± standard error, n=10, except where noted Reproductive tissue weights and spermatozoal data were analyzed for significance by Dunn's or Shirley's test, necropsy body weight data were analyzed for significance by Williams's test
[2] n=9
* Statistically significantly different (P≤0 05) from the control group
** Statistically significantly different (P≤0 01) from the control group

## TABLE C4 Summary of Estrous Cycle Characterization in Female B6C3F, Mice in the 13-Week Inhalation Study of Formic Acid

| Study Parameters[1] | 0 ppm | 8 ppm[2] | 32 ppm | 128 ppm |
|---|---|---|---|---|
| **Necropsy body weight (g)** | 32 7 ± 0 7 | 33 0 ± 0 9 | 31 9 ± 0 7 | 25 3 ± 0 3** |
| **Estrous cycle length (days)** | 4 00 ± 0 00 | 4 17 ± 0 12 | 4 00 ± 0 00 | 4 40 ± 0 15 |
| **Estrous stages as % of cycle** | | | | |
| Diestrus | 30 8 | 30 0 | 25 8 | 31 7 |
| Proestrus | 25 0 | 25 0 | 25 0 | 21 7 |
| Estrus | 27 5 | 25 8 | 27 5 | 27 5 |
| Metestrus | 16 7 | 19 2 | 21 7 | 19 2 |

[1] Data presented as mean ± standard error, n=10 Estrous cycle lengths are not significant by Dunn's test or Shirley's test By multivariate analysis of variance (MANOVA), dosed groups do not differ significantly from controls in the relative length of time spent in the estrous stages
[2] For 1/10 animals at this dose, estrous cycle length exceeded 12 days and the data was not included in the mean
** Statistically significantly different (P≤0 01) from the control group by William's test

# APPENDIX D

# Genetic Toxicology

**TABLE D1 Mutagenicity of Formic Acid in *Salmonella typhimurium*[1]**

| Strain | Dose (µg/plate) | Revertants/plate[2] -S9 Trial 1 | -S9 Trial 2 | + hamster S9 10% | + hamster S9 30% | + rat S9 10% | + rat S9 30% |
|---|---|---|---|---|---|---|---|
| TA100 | 0 | 133 ± 2.7 | 151 ± 11.0 | 171 ± 3.2 | 159 ± 9.2 | 162 ± 5.1 | 162 ± 9.1 |
| | 10 | | 164 ± 8.4 | 175 ± 3.8 | | 157 ± 12.2 | |
| | 33 | 132 ± 7.1 | 152 ± 10.1 | 160 ± 9.8 | 154 ± 4.3 | 162 ± 9.6 | 170 ± 3.1 |
| | 100 | 131 ± 3.5 | 157 ± 13.3 | 161 ± 5.8 | 139 ± 3.6 | 167 ± 5.7 | 161 ± 9.5 |
| | 333 | 138 ± 0.3 | 153 ± 9.8 | 175 ± 5.2 | 138 ± 7.5 | 170 ± 8.5 | 151 ± 9.0 |
| | 1000 | 137 ± 6.1 | 125 ± 15.3 | 165 ± 15.0 | 111 ± 3.5 | 157 ± 7.9 | 112 ± 10.7 |
| | 3333 | 68 ± 9.7[3] | | | 81 ± 4.6[3] | | 46 ± 20.2[3] |
| Trial summary | | Negative | Negative | Negative | Negative | Negative | Negative |
| Positive control[4] | | 319 ± 10.5 | 316 ± 3.5 | 657 ± 5.1 | 399 ± 4.9 | 599 ± 23.1 | 447 ± 17.5 |
| TA1535 | | | | | | | |
| | 0 | 12 ± 0.7 | 26 ± 2.4 | 9 ± 1.7 | 8 ± 2.5 | 11 ± 1.0 | 18 ± 1.2 |
| | 10 | | 21 ± 2.7 | 11 ± 1.3 | | 13 ± 2.6 | |
| | 33 | 16 ± 1.7 | 15 ± 0.9 | 10 ± 0.9 | 9 ± 2.3 | 16 ± 0.7 | 20 ± 4.3 |
| | 100 | 13 ± 0.9 | 19 ± 1.2 | 11 ± 1.2 | 12 ± 2.6 | 14 ± 2.6 | 15 ± 0.9 |
| | 333 | 8 ± 0.7 | 13 ± 0.3 | 11 ± 1.8 | 12 ± 1.7 | 10 ± 1.0 | 16 ± 0.6 |
| | 1000 | 13 ± 2.3 | 13 ± 0.6 | 12 ± 1.2[3] | 7 ± 1.2[3] | 10 ± 2.1 | 7 ± 0.7[3] |
| | 3333 | 11 ± 1.7 | | | 4 ± 2.1[3] | | 3 ± 1.5[3] |
| Trial summary | | Negative | Negative | Negative | Negative | Negative | Negative |
| Positive control | | 506 ± 24.1 | 332 ± 10.2 | 180 ± 18.4 | 340 ± 22.8 | 214 ± 27.7 | 60 ± 2.3 |

| Strain | Dose (µg/plate) | Revertants/plate -S9 Trial 1 | -S9 Trial 2 | + hamster S9 10% Trial 1 | + hamster S9 30% Trial 1 | + hamster S9 30% Trial 2 | + rat S9 10% Trial 1 | + rat S9 30% Trial 1 |
|---|---|---|---|---|---|---|---|---|
| TA97 | 0 | 144 ± 9.3 | 175 ± 14.6 | 207 ± 11.1 | 172 ± 4.1 | 204 ± 19.6 | 213 ± 5.9 | 178 ± 15.4 |
| | 10 | | 181 ± 7.1 | 213 ± 9.4 | | | 211 ± 9.5 | |
| | 33 | 179 ± 9.8 | 182 ± 9.2 | 214 ± 9.7 | 150 ± 6.6 | 214 ± 7.8 | 214 ± 3.5 | 191 ± 9.4 |
| | 100 | 179 ± 6.0 | 180 ± 10.5 | 206 ± 1.8 | 167 ± 34.0 | 213 ± 12.5 | 201 ± 24.8 | 182 ± 4.4 |
| | 333 | 165 ± 8.1 | 179 ± 9.5 | 222 ± 2.1 | 213 ± 13.0 | 196 ± 16.2 | 203 ± 10.4 | 188 ± 17.6 |
| | 1000 | 122 ± 2.7 | 185 ± 11.9 | 170 ± 8.6 | 146 ± 14.6 | 158 ± 10.7[3] | 153 ± 5.2 | 109 ± 7.2 |
| | 3333 | 65 ± 6.1 | | | 36 ± 10.2[3] | 65 ± 6.7[3] | | 85 ± 17.1[3] |
| Trial summary | | Equivocal | Negative | Negative | Equivocal | Negative | Negative | Negative |
| Positive control | | 415 ± 11.2 | 403 ± 11.1 | 463 ± 15.8 | 368 ± 24.9 | 451 ± 19.6 | 449 ± 10.1 | 454 ± 25.6 |

**TABLE D1 Mutagenicity of Formic Acid in *Salmonella typhimurium* (continued)**

| Strain | Dose (µg/plate) | Revertants/plate[2] | | | | | |
|---|---|---|---|---|---|---|---|
| | | -S9 | | + hamster S9 | | + rat S9 | |
| | | Trial 1 | Trial 2 | 10% | 30% | 10% | 30% |
| TA98 | 0 | 24 ± 2 6 | 24 ± 0 3 | 42 ± 2 1 | 32 ± 0 3 | 36 ± 3 7 | 40 ± 1 5 |
| | 10 | | 21 ± 2 3 | 18 ± 0 6 | | 28 ± 5 2 | |
| | 33 | 24 ± 2 0 | 17 ± 1 0 | 22 ± 2 8 | 31 ± 1 2 | 33 ± 5 4 | 35 ± 4 0 |
| | 100 | 24 ± 1 8 | 20 ± 2 0 | 42 ± 9 4 | 26 ± 0 3 | 30 ± 4 8 | 31 ± 1 5 |
| | 333 | 21 ± 3 5 | 14 ± 0 3 | 33 ± 4 0 | 26 ± 0 6 | 23 ± 1 5 | 23 ± 2 3 |
| | 1000 | 21 ± 2 3 | 20 ± 2 0 | 35 ± 2 2 | 25 ± 1 3 | 24 ± 3 5 | 25 ± 2 0 |
| | 3333 | 18 ± 0 0[3] | | | 11 ± 1 5 | | 7 ± 4 0[2] |
| Trial summary | | Negative | Negative | Negative | Negative | Negative | Negative |
| Positive control | | 380 ± 5 6 | 423 ± 27 7 | 202 ± 16 0 | 147 ± 9 3 | 202 ± 10 9 | 83 ± 7 3 |

[1] Study performed at SRI, International The detailed protocol and these data are presented in Zeiger *et al* (1987)
[2] Revertants are presented as mean ± the standard error from three plates
[3] Slight toxicity
[4] The positive controls in the presence of metabolic activation were 4-nitro-o-phenylenediamine (TA98), sodium azide (TA100 and TA1535), and 9-aminoacridine (TA97) The positive control for metabolic activation with all strains was 2-aminoanthracene