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DRAFT REPORT

SUPPORT FOR CHEMICAL NOMINATION AND SELECTION PROCESS OF THE NATIONAL TOXICOLOGY PROGRAM

EXECUTIVE SUMMARY OF DATA

CINNAMALDEHYDE

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Submitted to:

NATIONAL TOXICOLOGY PROGRAM

Submitted by:

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The information contained herein is based on data from current published literature and is believed to be accurate. However, no warranty is expressed or implied regarding the accuracy of these data or the results to be obtained from the use thereof.

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I. INTRODUCTION

Exposure to Cinnamaldehyde results primarily from its widespread use as a flavor and fragrance ingredient in food, beverages, medical products, cosmetics and perfumes. There are numerous reports in the literature describing cases of skin sensitization reactions resulting from both occupational and consumer exposure to Cinnamaldehyde. This compound has also been found to cause severe skin irritation following acute exposure.

There are conflicting reports concerning the mutagenicity of Cinnamaldehyde. Cinnamaldehyde has been found to be mutagenic to *Bacillus subtilis*, *Drosophila melanogaster*, Chinese hamster ovary cells, mouse leukocytes, hamster fibroblasts and *Salmonella* strain TA100. However, Cinnamaldehyde has also been found, by other authors, to be non-mutagenic to this strain of *Salmonella* and is reportedly non-mutagenic in other test systems. In addition, there are conflicting reports concerning the teratogenicity of this compound. In one study, Cinnamaldehyde was found to induce limb malformations in chick embryos. Although there are no data available which associate Cinnamaldehyde with carcinogenic effects in animals or humans, the transforming capacity of this compound has been demonstrated *in vitro*. In addition, two related compounds, 3, 4, 5-Trimethoxy Cinnamaldehyde and Cinnamyl Anthranilate, have been found to be animal carcinogens. Because Anthranilic Acid was observed to be non-carcinogenic, it is believed that the Cinnamyl moiety may play a role in the carcinogenicity of Cinnamyl Anthranilate.

Based on both the concern about the possible carcinogenicity of the Cinnamyl moiety, and the importance of Cinnamaldehyde as a flavor ingredient in food, the Food and Drug Administration (FDA) has nominated Cinnamaldehyde as its priority chemical for fiscal year 1989. Cinnamaldehyde was originally nominated to the National Toxicology Program (NTP) in 1979, at which time the Chemical Nomination and Selection Committee (currently the Chemical Evaluation Committee) recommended that this compound be selected for testing. However, because of scheduled budget cuts and subsequent reallocation of resources, toxicological studies on Cinnamaldehyde were not performed.

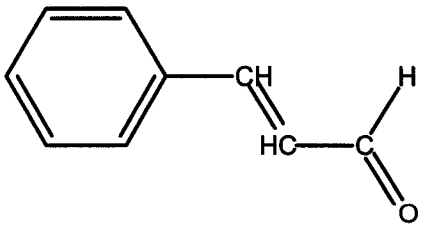
II. CHEMICAL AND PHYSICAL PROPERTIES

A. Synonyms: Cinnamaldehyde (8CI)
Abion Cinnamaldehyde
Acrolein 3-Phenyl
A13-00473
Benzylideneacetaldehyde
Cassia aldehyde
Caswell No. 221A
Cinnamal
Cinnamic Aldehyde
Cinnamyl Aldehyde
EPA Pesticide Chemical Code 040506
HSDB 209
NCI-C56111
Zimtaldehyde
2-Propenal, 3-phenyl (9CI)
3-Phenyl-2-Propenal
3-Phenyl-2-Propenaldehyde
3-Phenylacrolein
3-Phenylpropenal.

B. CAS NO: 104-55-2

C. Molecular Formula: C_9H_8O

D. Structure:



E. Molecular Weight: 132.15

F. Physical Properties:

1. Appearance/Odor: Yellowish (darkens on exposure to light and air) [30], oily/strong odor of cinnamon [76]
2. Physical State: Liquid (thickens on exposure to air [64] and light [30])
3. Freezing Point: -7.5°C (18.5°F) [73, 76]

4. Boiling Point:	76.1°C (168.9°F) @ 1 mm Hg 105.8°C (222.4°F) @ 5 mm Hg 120.0°C (248.0°F) @ 10 mm Hg 135.7°C (276.0°F) @ 20 mm Hg 152.2°C (305.9°F) @ 40 mm Hg 163.7°C (326.6°F) @ 60 mm Hg 177.7°C (351.8°F) @ 100 mm Hg 199.3°C (390.7°F) @ 200 mm Hg 222.4°C (432.3°F) @ 400 mm Hg 246.0°C (474.8°F) @ 760 mm Hg [76] (some decomposition) 248.0°C (478.4°F) [2] 253.0°C (487.4°F) (decomposes) [73]
5. Flash Point:	71°C (160°F) [2]
6. Vapor Density:	4.6 (air=1) [8]
7. Vapor Pressure:	1 mm Hg @ 76.1°C 40 mm Hg @ 152°C [71]
8. Specific Gravity:	1.048 to 1.052 @ 25°/25°C [76]
9. Refractive Index:	1.618 to 1.623 [76]
10. Solubility in Water:	Very slightly soluble [65] (dissolves in approximately 700 parts water [76])
11. Solubility in Organic Solvents:	Soluble in alcohol, ether, chloroform [73, 76], oils [76]
12. Log Octanol/Water Partition Coefficient:	1.88 [71]
13. Other:	• Volatile with steam [76]

- May ignite after a delay period in contact with NaOH [5, 65]
- When heated to decomposition emits acrid smoke and fumes [65]

III. PRODUCTION/USE

A. Production

1. Manufacturing Process

Cinnamaldehyde is manufactured by the condensation of Benzaldehyde and Acetaldehyde in the presence of Sodium Hydroxide [30, 64], Calcium Hydroxide, Hydrochloric Acid or Sodium Ethylate [47]. Other methods of synthesizing Cinnamaldehyde include hydrolysis of Cinnamyl Dichloride by cold water, oxidation of Cinnamyl Alcohol, treatment of (1-Chloroallyl) Benzene with Phosphorus Pentachloride and conversion of the resulting (2,3,3-Trichloropropyl) Benzene to Cinnamaldehyde, as well as the condensation of Styrene with Formylmethylaniline in the presence of Phosphorus Oxychloride [30].

2. Major Manufacturers

U.S. Manufacturers of Cinnamaldehyde include:

- Berje, Inc.
  5 Lawrence Street
  Bloomfield, New Jersey

- Chemical Dynamics Corp.
  Hadley Road
  South Plainfield, New Jersey

- CHEM-FLEUR, Inc.
  Newark, New Jersey

- D & O Chemicals, Inc.
  291 South Van Brunt Street
  Englewood, New Jersey
- Fritzsche Dodge & Olcott, Inc.
  East Hanover, New Jersey

- Givaudan Corporation, Chemicals Division
  Clifton, New Jersey
- Haarmann & Revmer Corp.

70 Diamond Road
Springfield, New Jersey

- NIPA Laboratories, Inc.
  3411 Silverside Road
  Wilmington, Delaware

- Penta Manufacturing Company
  P.O. Box 1448
  Fairfield, New Jersey

- Quest International Fragrances USA Inc.
  400 International Drive
  Mount Olive, New Jersey

- Universal Oil Products Company
  East Rutheford, New Jersey [7,47]

3. Volume

In 1977, 911,730,000 grams of Cinnamaldehyde were produced in the United States as reported by the United States International Trade Commission. Between 1978 and 1989, production data on this compound were not published.

There are no export or import data available in the current literature for Cinnamaldehyde. The following import data have been reported for Cinnamon Oil: in 1972, 1.85×10^7 grams of Cinnamon Oil were imported to the United States. In 1975, 1.9×10^7 grams were reportedly imported [47].

B. Use

Cinnamaldehyde is used primarily in the flavor and fragrance industries for imparting a cinnamon flavor and/or fragrance to various types of foods, beverages, medical products, and perfumes. This chemical is used in the liquor industry for flavoring liqueurs and cordials. Cinnamaldehyde has also been used as a rubber reinforcing agent, a filtering agent, an attractant for termites, a corrosion inhibitor for sulfuric acid baths to clean galvanized iron and zinc, as an emulsion fog inhibitor for photographic

film, as a component of photographic hardening bleaches, and in electroplating baths [30,47].

Approximately 5.00 x 10^8 grams of Cinnamaldehyde were used in 1978 as a flavor and fragrance ingredient. This figure, based on sales in the United States, was reported by the United States International Trade Commission [3].

IV. EXPOSURE/REGULATORY STATUS

A. Consumer Exposure

Consumer exposure to Cinnamaldehyde results primarily from the widespread use of this compound as a flavor and fragrance ingredient in food, beverages, medical products, cosmetics and perfumes. Cinnamaldehyde is reportedly used in foods and beverages at the following levels:

Fruits and Vegetables	6400	ppm	
Chewing Gum	4900	ppm	
Baked Goods	3500	ppm	
Breakfast Cereals	2200	ppm	
Baby Food	2000	ppm	
Candy	700	ppm	
Meats	60	ppm	
Condiments	20	ppm	
Non-Alcoholic Beverages	9.0	ppm	
Ice Cream	7.2	ppm	[12,47]

B. Occupational Exposure

Occupational exposure to Cinnamaldehyde has occurred in the fragrance, cosmetic [6], beverage, and food industries [69]. Cinnamaldehyde exposure among cinnamon workers [70], hairdressers [34], and bakers [24] has been reported. Occupational exposure to Cinnamaldehyde has also occurred in deodorant manufacturing plants [52] and could presumably occur during the manufacture of medical products.

Data from the National Occupational Exposure Survey (NOES), conducted by the National Institute for Occupational Safety and Health (NIOSH) between 1981 and 1983, indicated that 2,574 workers, including 1,828 female employees, were potentially exposed to Cinnamaldehyde in the workplace. The NOES data base does

not contain information on the frequency, level, or duration of exposure to workers of any of the chemicals listed therein.

C. Environmental Exposure

Cinnamaldehyde occurs naturally in Chinese cinnamon oil from the leaves and twigs of *Cinnamonum cassia* [30]. Cinnamaldehyde is also found in the essential oils of Ceylon and Madagascar cinnamon leaves as well as in Ceylon, Seychelles and Japanese Cinnamon bark. Cinnamaldehyde is present in the essential oils of hyacinth, myrrh, Bulgarian rose and patchouli [12]. This compound also occurs naturally in the fungus *Stereum subpileatum* [7].

Cinnamaldehyde has not been found in United States drinking water supplies or industrial effluents [7].

D. Regulatory Status

- Food and Drug Administration (FDA), section number 121.101, GRAS (generally recognized as safe); limited to use as a synthetic flavor/adjuvant [47].

- Code of Federal Regulations, 21 CFR 182.60; Synthetic Flavoring Substances and Adjuvants, GRAS (generally recognized as safe) for its intended use when used in accordance with good manufacturing or feeding practice [54].

- Code of Federal Regulations, 15 CFR 399.2 Supp. 1; Commodity Control List requiring a valid license for export [55].

- Flavor and Extract Manufacturer's Association (FEMA) Number 2286 [12].

- Reported in the Environmental Protection Agency (EPA), Toxic Substances Control Act (TSCA) Inventory, 1989 [65].

- Joint FAO/WHO Expert Committee on Food Additives, temporary acceptable daily intake (ADI) of up to 700 μg per kg body-weight [79].

- Canadian Workplace Hazardous Materials Information System (WHMIS), Ingredient Disclosure List; Canadian IDL: 0.1% concentration [62].

- There is no OSHA permissible exposure limit (PEL) or ACGIH recommended threshold limit value (TLV).

V. TOXICOLOGICAL EFFECTS

A. Acute

1. Animal Data

Exposure to Cinnamaldehyde had been found to affect the central nervous, cardiovascular, and digestive systems. This compound has also reportedly caused contact urticaria, diarrhea, depression and coma in animals following acute exposure.

Cinnamaldehyde has been found to have both inhibitory and excitatory effects on the central nervous system of mice. Intraperitoneal administration of this compound at doses higher than 100 mg/kg was observed to cause a transient excitation (running fit) followed by a depression in activity [72].

Cinnamaldehyde has been observed to affect the cardiovascular system of dogs and guinea pigs. Intravenous administration of 5-10 mg/kg to male and female Mongrel dogs was found to reduce blood pressure and increase respiratory rate and femoral blood flow. Heart rate was observed to increase simultaneously with the fall in blood pressure, and thereafter to return to baseline.

A fall in blood pressure was also observed in male guinea pigs following intravenous administration of Cinnamaldehyde at a dose of 1 mg/kg. Heart rate was lowered by 15 percent following administration of this compound at a dose of 5 mg/kg, while femoral blood flow was observed to increase. In experiments using isolated guinea pig hearts, Cinnamaldehyde administered at doses ranging from 50 to 500 μg was found to increase heart beat rate and to induce arrhythmias at does greater than 250 μg.

Cinnamaldehyde has also been observed to affect the digestive systems of rats and mice. In male, dd mice, Cinnamaldehyde was found to have an inhibitory effect on intestinal propulsion following intraperitoneal administration at a dose of 250 mg/kg. In addition, Cinnamaldehyde was observed to decrease stress-induced gastric erosion at an intraperitoneal dose of 250 mg/kg. In male, Wistar rats, this

compound was found to inhibit spontaneous gastric contraction at an intravenous dose of 5 mg/kg. Oral administration of Cinnamaldehyde at a dose of 500 mg/kg reportedly increased biliary excretion. Cinnamaldehyde did not change the pH value of gastric perfusate at intravenous doses up to 10 mg/kg [18].

Cinnamaldehyde has been found to induce nonimmunologic contact urticaria in guinea pigs, rats and mice, with symptoms ranging from slight erythema to extensive local erythema and edema accompanied by tingling, burning and itching, following application of a 20% solution to the earlobes. The thickness of the earlobes was measured before, during and after the application. Maximal ear swelling was observed 20 to 50 minutes after the application of Cinnamaldehyde and reportedly decreased during the three-hour observation period [32].

Acute expose to Cinnamaldehyde has been found to cause diarrhea and depression in rats. High, acute doses of this compound have induced coma in rats [48]. Acute systemic toxicity values for Cinnamaldehyde are presented in Table 1.

2. Human Data

Acute exposure to Cinnamaldehyde may result in skin, eye [58], respiratory [47] and gastrointestinal irritation. Systemic effects from acute exposure are believed to be limited [16]. Acute toxicity data available for Cinnamaldehyde is restricted primarily to this compound's effect on the skin.

Cinnamaldehyde has been found to cause severe skin irritation followingapplication of 40 mg for 48 hours [48]. A 3 percent solution of Cinnamaldehyde in petrolatum was not found to cause skin irritation after a 48 hour closed-patch test on humans. However, an 8 percent solution was found to be severely irritating to the skin, and the concentration had to be reduced to 2 percent for the test to be completed [56].

The acute toxicity of Cinnamaldehyde has been assessed *in vitro* using cultured human KB cells. A dose response curve was obtained following a 72-hour, KB cell exposure to various concentrations of Cinnamaldehyde. The 72-hour ID_{50}[1] was determined to be 19.50 μg/ml. This was compared to a 72-hour ID_{50} value of 70.0 μg/l for *Saccharo- myces cerevisie* tested under identical conditions[43].

2. Case Reports

Cinnamaldehyde has been found to cause contact urticaria in children. Children being treated for contact urticaria were patch tested for skin reaction to a variety of fragrances and food additives. Children who developed palpable pruritic erythema 20 minutes after exposure were considered positive for contact urticaria reactions. Twelve out of 125 children reportedly had a positive patch test result for Cinnamaldehyde [60].

TABLE 1

Acute Systemic Toxicity Values for Cinnamaldehyde

Route	**Species**	**Dose**	**Reference**
Oral	Rat	**LD_{50}=2220 mg/kg** Toxic Effects: behavioral (somnolence); gastrointestinal (hypermotility diarrhea)	[48]
Oral	Rat	**LD_{50}=3350 mg/kg**	[56]
Oral	Mouse	**LD_{50}=2225 mg/kg** Toxic effects: behavioral (convulsions or effect on seizure threshold; ataxia), respiratory stimulation	[48]
Oral	Guinea Pig	**LD_{50}=1160 mg/kg** Toxic effects: behavioral (coma at higher doses)	[48]
Intraperitoneal	Mouse	**LD_{50}=200 mg/kg** Toxic effects: none	[8,48]
Parenteral	Mouse	**LD_{LO}=200 mg/kg** Toxic effects: not reviewed	[48]
Intravenous	Mouse	**LD_{50} =75 mg/kg** Toxic effects: none noted	[48]
Dermal	Rabbits	**LD_{50}=0.42-0.84 mg/kg** Toxic effects: not reviewed	[56]

B. Subchronic/Chronic

1. Animal Data

The data available in the literature concerning the subchronic and chronic toxicology of Cinnamaldehyde in animals primarily concerns the sensitizing effect of this chemical. The contact sensitization potential of Cinnamaldehyde has been tested in female, Balb/C mice maintained on a diet supplemented with vitamin A acetate[2]. The sensitization protocol included an induction period of two weeks followed by a total of six topical applications of a 30 percent Cinnamaldehyde solution to the shaved abdomen and thorax. This was followed one week later by a topical challenge of 15 percent Cinnamaldehyde to both ears. Ear thickness was measured before the challenge as well as 24 and 48 hours after the challenge. The percent increase in ear thickness was determined, and the statistical significance of increased ear thickness was assessed by the Mann Whitney U test. A compound was classified as a sensitizer if the Mann Whitney test was significant at P# 0.01 or the Mann Whitney test was significant at P not > 0.05, and in addition 2 animals had increases in ear thickness twice that of the highest control increase.

One mouse from the group of ten tested was found to have an increase in ear thickness 24 hours after the challenge that was 100 percent greater than the highest increase in the control group, while six mice had increases in ear thickness after the challenge that were determined to be 50 percent greater than the highest increase in the control group. The Mann Whitney test was found to be significant at P< 0.01, classifying Cinnamaldehyde as a contact sensitizer [36].

Effects observed following dietary administration of Cinnamaldehyde to male and female rats over a sixteen week period at a concentration of 10,000 ppm include slight hyperkeratosis of the squamous portion of the stomach lining and slight swelling of the hepatic cells. When administered at doses of 2 mg on alternate days to two generations of rats for 223 and 210 days respectively, Cinnamaldehyde was found to cause an increase in liver weight by 20 percent in the first generation and 22 percent in the second.

The maximum tolerated dose (MTD) of Cinnamaldehyde defined as the maximum single dose tolerated by a group of five mice following six intraperitoneal injections over a two week period was determined to be 0.25 g/kg [56].

2. Case Reports

Numerous case reports describe the skin sensitization potential of Cinnamaldehyde in humans. Skin sensitization has been found to occur following both occupational and consumer exposure to this compound. In some cases, the skin sensitization caused by Cinnamaldehyde has been found to be permanent [58]. The following cases of chronic contact dermatitis from occupational exposure to Cinnamaldehyde are presented in the literature:

A case of allergic contact dermatitis from exposure to Cinnamaldehyde at an air freshener manufacturing plant has been reported. A 43 year old man who had no history of non-occupational exposure to perfumed products developed an itchy eruption on his fingertips which began one month after he began working at the plant. The eruption was confined to his hands and consisted of erythematous scaling patches with indistinct borders on the fingertips and the dorsal surfaces of both hands. In his job, the employee added various fragrances to a dispensing machine that subsequently applied the fragrances to pads used to make household air freshening devices. In addition, the employee served as a maintenance person and was frequently exposed to full-strength perfume concentrates from malfunctioning equipment.

Patch tests were performed on the employee using the European Standard Patch Test Series. The worker only developed an allergic response to Cinnamaldehyde. The eight fragrance concentrates to which the worker was exposed were subsequently analyzed for their Cinnamaldehyde content; three of the eight fragrances were found to have detectable levels of Cinnamaldehyde. It was concluded that the allergic contact dermatitis most likely resulted from repeated skin contamination with full-strength perfume concentrates [52].

An increased incidence of fragrance-related occupational dermatitis among a group of coal miners being treated for eczematous skin problems has been documented. Thirty five miners, 55 male non-miners and 30 female non-miners were patch tested over a period of eighteen months using the International Contact Dermatitis Research Group (ICDRG) Standard Series. Forty-five percent of the coal miners were found to be fragrance sensitive while 20 percent of the male, and 13 percent of the female non-miners had positive patch test results. Cinnamaldehyde

reportedly caused the highest number of positive responses among the male miners and the male non-miners tested; 14 of the miners and 7 of the non-miners developing positive patch test results after 96 hours. The increased incidence of allergic contact dermatitis among the coal workers is believed to be related to a highly perfumed body lotion used at the coal mine [15].

A high incidence of occupationally-related allergic skin reactions was also reported among factory workers in a Danish spice manufacturing plant. Almost all of the workers exposed to high concentrations of Cinnamaldehyde during the manufacture of cinnamon spice substitutes developed sensitivity to Cinnamaldehyde [56].

During an eight-year study, 66 hairdressers who were being treated by dermatologists for contact dermatitis were patch tested to the North American Contact Dermatitis Group Standard Screening Trays and to a hairdressers' screening tray. Cinnamaldehyde was found to produce allergic skin reactions in 1.5% of the hairdressers tested [34].

The following cases of chronic contact dermatitis from consumer exposure (toothpaste, cosmetics, fragrances) to Cinnamaldehyde are reported in the literature:

Over a six-month period, a 25 year-old woman reportedly developed perioral leukoderma caused by a Cinnamaldehyde-containing toothpaste. The leukoderma around the woman's mouth began at the oral commissures and had spread above and below the lips. Porcelain-white depigmentation of the skin lateral to the oral commissures was observed. In addition, leukoderma of the perioral skin adjacent to the borders of her lips was marginated by a thin border of hyperpigmentation. Patch testing was performed using the routine screening series of the North American Contact Dermatitis Group (NACDG) which included a 2 percent solution of Cinnamaldehyde in petrolatum. A positive (2+) papular reaction to Cinnamaldehyde was observed 48 and 96 hours after exposure.
It was subsequently determined that two years before the onset of the leukoderma, the woman had begun using a Cinnamaldehyde-containing toothpaste. Six months after she switched to a non-Cinnamaldehyde-containing toothpaste, the perioral leukoderma almost completely disappeared [41].

Consumer exposure to Cinnamaldehyde has reportedly caused chronic cheilitis in an 82 year-old woman who had been using both a Cinnamaldehyde-containing toothpaste and a sunscreen lipstick. The woman's symptoms consisted of cracking, swelling and peeling lips, but no cutaneous lesions were observed. Patch testing with the standard fragrances and preservative series utilizing ICDRG standard techniques resulted in a positive reaction only to Cinnamaldehyde. When the woman stopped using the Cinnamaldehyde-containing toothpaste and lipstick her symptoms cleared [35].

Two case reports of cosmetic intolerance among persons being treated for chronic contact dermatitis are described in the literature. In one study, 5202 patients were patch tested using the Belgian Tri-Contact Patch Test Series. Eight percent of the total test population reacted positively to cosmetic patch tests. Perfumes were the principal allergens observed in the group of patients who suffered from pure allergies to cosmetics (156 patients). Of these cases, 5.1 percent were attributed to Cinnamaldehyde.

In the second study, 182 patients suspected of suffering from contact sensitization to cosmetics were patch tested using the standard tray of the ICDRG as well as 22 fragrance raw materials. Cinnamaldehyde was found to produce positive results in 3.7 percent of the patients tested [38].

Over a period of more than three years, 2826 patients at the Göttingen University Hospital for Skin Diseases were tested for skin sensitivity to Cinnamaldehyde. Only 0.74 percent of the patients (21) reacted positively to Cinnamaldehyde. It was noted by the authors that in countries other than Germany, especially England and the United States, allergy to Cinnamaldehyde occurs more frequently. The discrepancy is presumably a result of the variation in consumer exposure to Cinnamaldehyde between different countries [67].

C. Carcinogenicity

1. Animal Data

There are limited data available concerning the carcinogenicity of Cinnamaldehyde in animals. Cinnamaldehyde has been tested for its hepatocarcinogenicity in male,

$B6C3F_1$ mice following injection on days 1, 8, 15 and 22 prior to weaning. The concentration of Cinnamaldehyde injected per dose was in the ratio of 1:2:4:12 respectively, for a total dose of 4.8 μmol per mouse. Cinnamaldehyde showed no hepatocarcinogenic activity at the dose levels tested [77].

The remaining information on the carcinogenic effects of this compound concerns its transforming capacity. The transforming potency of Cinnamaldehyde has been demonstrated by *in vitro* studies using Chinese hamster epithelial cells (CH-B241). The CH-B241 cells were treated with sublethal doses of Cinnamaldehyde (10nM), and the surviving cells were cultivated until they acquired characteristics typically associated with transformed cells; namely 1.) an increase in saturation density in the monolayer culture, 2.) an increase in plating efficiency at a low serum level, or 3.) an increase in colony forming efficiency in soft agar medium. The treated CH-B241 cells that met these *in vitro* criteria were subsequently analyzed for their ability to induce neoplastic transformation. This was achieved by subcutaneous injection of 1×10^6 cells into a suprascapular region of male, nude mice (BALB/C, JCL, NuNu).

Formation of nodules at the injection site was observed in six out of seven mice treated with Cinnamaldehyde-transformed cells. One mouse produced nodules in the liver and spleen, indicating metastasis. The nodules were first palpable between days 91 and 237 after injection, after which they grew slowly to 2 cm in diameter until day 311. When the tumors at the injection site reached 2 cm in diameter, the animals were sacrificed and the tumors were removed for histological examination. Microscopic examination revealed that the tumors were malignant and consisted of cells with random shaped nuclei and a high frequency of mitosis. Karyotype analysis demonstrated that approximately 45 percent of the tumor cells were polyploid.

In addition, tumors were aseptically removed from the mice, and cells from the tumors were re-injected into mice in order to assess serial transplantability. Tumor formation was observed at the injection site in all animals tested within a considerably shorter latent period (17 to 114 days) than that observed following the primary inoculation. Metastasis of the spleen was observed in three out of four animals injected with tumor cells from the Cinnamaldehyde-treated mice.

Although the *in vitro* transforming potency of Cinnamaldehyde was demonstrated, the induction mechanism is unclear. Direct or indirect interaction with genetic material is presumably involved because considerable structural chromosomal aberrations, including chromosome and/or chromatid breaks, were observed [27, 29].

Cinnamaldehyde has been tested for its capacity to enhance the transformation of Syrian hamster embryo cells by Simian adenovirus, SA7. Various sub-lethal doses (0.01 mm, 0.02 mm, 0.05 mm, 0.09 mm, 0.19 mm) were diluted in cell culture medium and added to replicate dishes of Syrian hamster embryo cells for 20 hours. After 20 hours, the cells were rinsed and SA7 virus was absorbed for 3 hours. The number of colonies from Cinnamaldehyde and virus treated cells were determined. This number was divided by the number of colonies from virus inoculated control cells in order to determine the surviving fraction. The number of SA7 foci from 2 x 10^6 plated cells was determined and the enhancement ratio was calculated by dividing the transformation frequency of treated cells by the transformation frequency of the control cells. The Cinnamaldehyde-induced enhancement was found to be statistically significant (P#0.05 or P#0.01) at only one dose level 0.05mM (see Table 2). Therefore, based on standard classification criteria, it was concluded that there is "some evidence" that Cinnamaldehyde enhances viral transformation [21].

2. Human Data

There are no data available on the carcinogenicity of Cinnamaldehyde in humans. However, the *in vitro* transforming potency of this chemical has been studied. Cinnamaldehyde was not found to induce transformation of the human fibroblast cell line HAIN-55 following treatment with various concentrations ranging from 5-80 nM [29].

D. Mutagenicity/Genetic Toxicology

1. Animal Data

There are conflicting reports concerning the mutagenicity of Cinnamaldehyde. This compound has been found to be mutagenic to

TABLE 2
EFFECT OF CINNAMALDEHYDE ON SA7 TRANSFORMATION[3]

Concentration (mM)	Surviving fraction (%)	SA7 foci	Enhancement ratio
0.19	72	20	1.07
0.09	107	34	1.22
0.05	100	57**	2.19**
0.02	100	32	1.23
0.01	83	42	1.94*
0	100	26	1.00

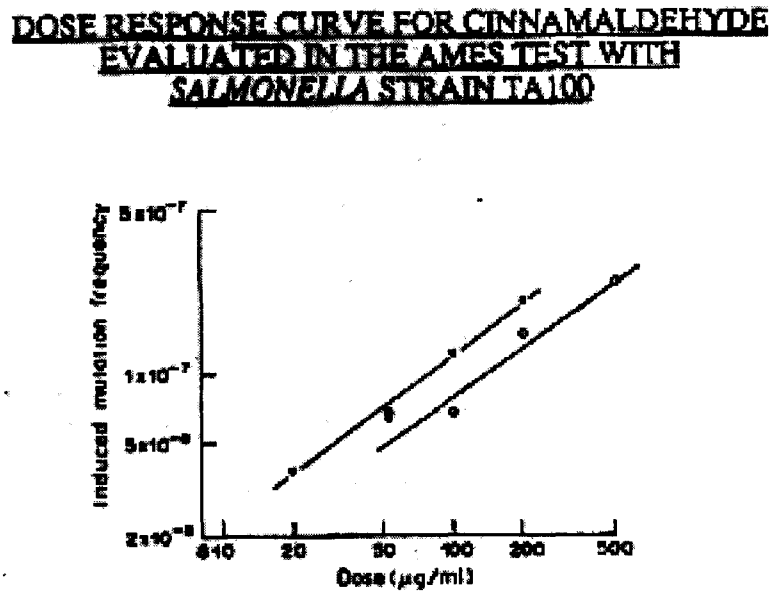
Bacillus subtilis, Drosophila melanogaster, Chinese hamster ovary cells, mouse leukocytes, hamster fibroblasts, and *Salmonella typhimurium* (strain TA100). However, other sources report that Cinnamaldehyde was non-mutagenic to rat hepatocytes, *Escherichia coli* and several strains of *Salmonella typhimrium*, including TA100.

Standard Ames reverse mutation assays were carried out using *Salmonella typhimurium* strains TA92, TA1535, TA100, TA1537, TA94 and TA98 in the presence and absence of liver microsome fraction Cinnamaldehyde was added at six different concentrations (10, 20, 50, 100, 200 and 500 µg/ml) per plate, and the number of revertant colonies was scored after incubation at 37°C for two days. Cinnamaldehyde induced 222 revertants at 0.5 mg/plate as compared to 146 in the control plates and 318 revertants (139 in the control) at 0.1 mg/plate in strain TA100 with and without metabolic activation, respectively (see Figure 1). Cinnamaldehyde was non-mutagenic in the other *Salmonella* strains tested [25].

Cinnamaldehyde has been found by other authors to be non-mutagenic to *Salmonella typhimurium* strains TA1535, TA1537, TA98 as well as TA100 in the presence and absence of metabolic activation [45, 59].

FIGURE 1

DOSE RESPONSE CURVE FOR CINNAMALDEHYDE EVALUATED IN THE AMES TEST WITH *SALMONELLA* STRAIN TA100



• Assay performed without S-9

o Assay performed with S-9

Cinnamaldehyde was also reportedly nonmutagenic to *S. typhimurium* strain TA104 in the absence of metabolic activation [40].

In order to detect its DNA-damaging potential, Cinnamaldehyde was tested in the spore rec- assay with *Bacillus subtilis* strains M45 (rec-) and H17 (rec+). The DNA damaging activity was assessed by growth inhibition zone measurements. Cinnamaldehyde was found to be mutagenic at a maximal dose of 10 µl per disk [80].

In addition, Cinnamaldehyde has been tested for its mutagenic activity in germ cells of *Drosophila melanogaster* using the sex linked recessive lethal mutation and the reciprocal translocation tests. Cinnamaldehyde was negative in the recessive lethal mutation test when tested by adult feeding methods. However, when tested by adult injection at 20,000 ppm, Cinnamaldehyde was found to

induce sex-linked recessive lethal mutations in meiotic and post-meiotic germ cell stages. Cinnamaldehyde was negative in the reciprocal translocation test [78].

Cinnamaldehyde has been found to induce chromosomal aberrations in Chinese hamster fibroblast cells at concentrations of 0.01 mg/ml after a 48 hour exposure and 0.015 mg/ml following a 24 or 48 hour exposure in the absence of metabolic activation. In order to obtain a quantitative evaluation of the clastogenic potential of Cinnamaldehyde, the D_{20}[4] and TR[5] values were calculated. Cinnamaldehyde was determined to be mutagenic at relatively low dose levels (D_{20}=0.01) and was found to have the highest TR value (TR=2133) among a total of 190 food additives tested. TR values are generally reported to be high for chemicals having carcinogenic potential in animals [25].
Cinnamaldehyde has been tested for its ability to induce sister chromatid exchange in Chinese hamster ovary cells in the presence and absence of metabolic activation. Cinnamaldehyde was found to be weakly positive in the sister chromatid exchange (SCE) test with and without metabolic activation, at a least effective concentration (LEC)[6] of 0.34 μg/ml (See Table 3). In the test system without metabolic activation, a low dose of mitomycin C was used as a "weak positive" control. In test system with metabolic activation, a low dose of cyclophosphamide was utilized. These "weak positive" controls were designed to give a small (20-40%) increase in SCEs and were included to assess the ability of the system to detect small increases in sister chromatid exchange. There was no evidence that Cinnamaldehyde induced chromosomal aberrations in Chinese hamster ovary cells [13].

TABLE 3

"Weakly Positive" Result in the SCE Test (-S 9)

Dose (μg/ml)	Total Chromosomes	Total SCE	SCE per cell
0.0000	1047	398	7.98
0.3400	1051	530	10.59*
1.0200	1050	697	13..94*

* Values are 20% above control level.

Cinnamaldehyde has been found to cause DNA inhibition in mouse leukocytes *in vitro* when tested in the L5178Y TK +/- Mouse Lymphoma Forward Mutation

Assay assay. In the presence and absence of activation, Cinnamaldehyde reportedly induced a "questionable" mutagenic response [57]. No additional information was provided.

Cinnamaldehyde was not mutagenic in an *in vivo* test for the induction of unscheduled DNA synthesis in rat hepatocytes following administration by gavage [42]. In addition, Cinnamaldehyde did not cause micronucleus induction in an *in vivo* micronucleus test with bone marrow mouse cells [22].

2. Human Data

There are no data available in the literature concerning the mutagenicity of Cinnamaldehyde in humans.

E. Teratology/Reproductive Toxicology

1. Animal Data

The reproductive effects of Cinnamaldehyde have been examined in rats and mice, and in both species Cinnamaldehyde was found to be negative for all parameters tested. However, there are conflicting reports concerning the teratogenic effects of Cinnamaldehyde.

Teratogenic parameters have been evaluated following administration of Cinnamaldehyde to pregnant, CD-1 mice at a dose level of 1,200 mg/kg/day in corn oil. Parameters included the number of females producing viable litters, the number of females with resorbed or nonviable litters, the number of proven pregnant females and the reproductive index[7]. In addition, group litter and viability data were evaluated, including the number of live pups per litter, the number of dead pups per litter, the litter weight and the mean pup weight. No significant differences from the control group were observed in any of the criteria examined [23].

In another study, CD-1 mice were dosed by gavage at 1,200 mg/kg/day of Cinnamaldehyde during mid-pregnancy. Litter size, birth weight, neonatal growth and survival to postnatal day three were recorded as indices of potential developmental toxicity. Both the maternal response variables and the neonatal

response variables tested were not found to differ significantly from the control [20].

Cinnamaldehyde was not found to affect body weight gain, reproductive ability, or the development and viability of offspring following administration of 2 mg on alternate days to two generations of rats for 223 and 210 days respectively [56]. Suprablastodermic administration of a single dose of Cinnamaldehyde to 3 day-old chick embryos (white Leghorn x Rhode Island red strain) was reportedly teratogenic. The Optimal Teratogenic Dose (OTD)[8] was found to be 0.50 μM per embryo. At this concentration, the most common teratogenic effects observed included limb malformations, primarily limb size reduction. Malformations of the axial skeleton including spina bifida, anoura (tail absence) or haemisomia were noted in several cases [1].

2. Human Data

There are no data available in the literature concerning the reproductive or teratogenic effects of Cinnamaldehyde on humans.

F. Immunotoxicity

1. Animal Data

There are no data available in the literature concerning the Immunotoxicity of Cinnamaldehyde in animals.

2. Human Data

There are no data available in the literature concerning the immunotoxicity of Cinnamaldehyde in humans.

VI. CHEMICAL DISPOSITION

A. Animal Data

The elimination of Cinnamaldehyde has been studied in the female, Wistar rats after administration of 250 mg/kg daily for two weeks. Following this dosing regimen, two sulphur-containing metabolites were isolated from the urine and identified by synthesis, nuclear magnetic resonance (NMR) and mass spectrography as N-acetyl-S-(1-phenyl-3-hydroxypropyl) cysteine and N-acetyl-S-(1-phenyl-2-carboxy ethyl) cysteine in a 4:1 ratio. The total thioether excretion, calculated as a percentage of the dose of Cinnamaldehyde administered, was determined to be 14.8± 1.9%.

NMR spectra of the isolated mercapturic acids indicated that addition of a nucleophilic Glutathione anion occurred to the ß-carbon atom of the double bond of Cinnamaldehyde. At some stage during the conversion of the intermediate Glutathione conjugate of Cinnamaldehyde to a mercapturic acid, reduction of the carbonyl moiety to a hydroxy group occurred. In addition, a small portion of the carbonyl moieties were oxidized into a carboxylic group (see Figure 2) [9,10].

Cinnamaldehyde, which contains activated double bonds that are substrates for Glutathione S-Alkenetransferases, has been found to depress liver Glutathione levels markedly following intraperitoneal administration to rats at a dose of 0.5 ml/kg. Thirty minutes after administration, the Glutathione level had been reduced to 53 percent of the control, and after two hours, the Glutathione level had dropped to 35 percent of the control.

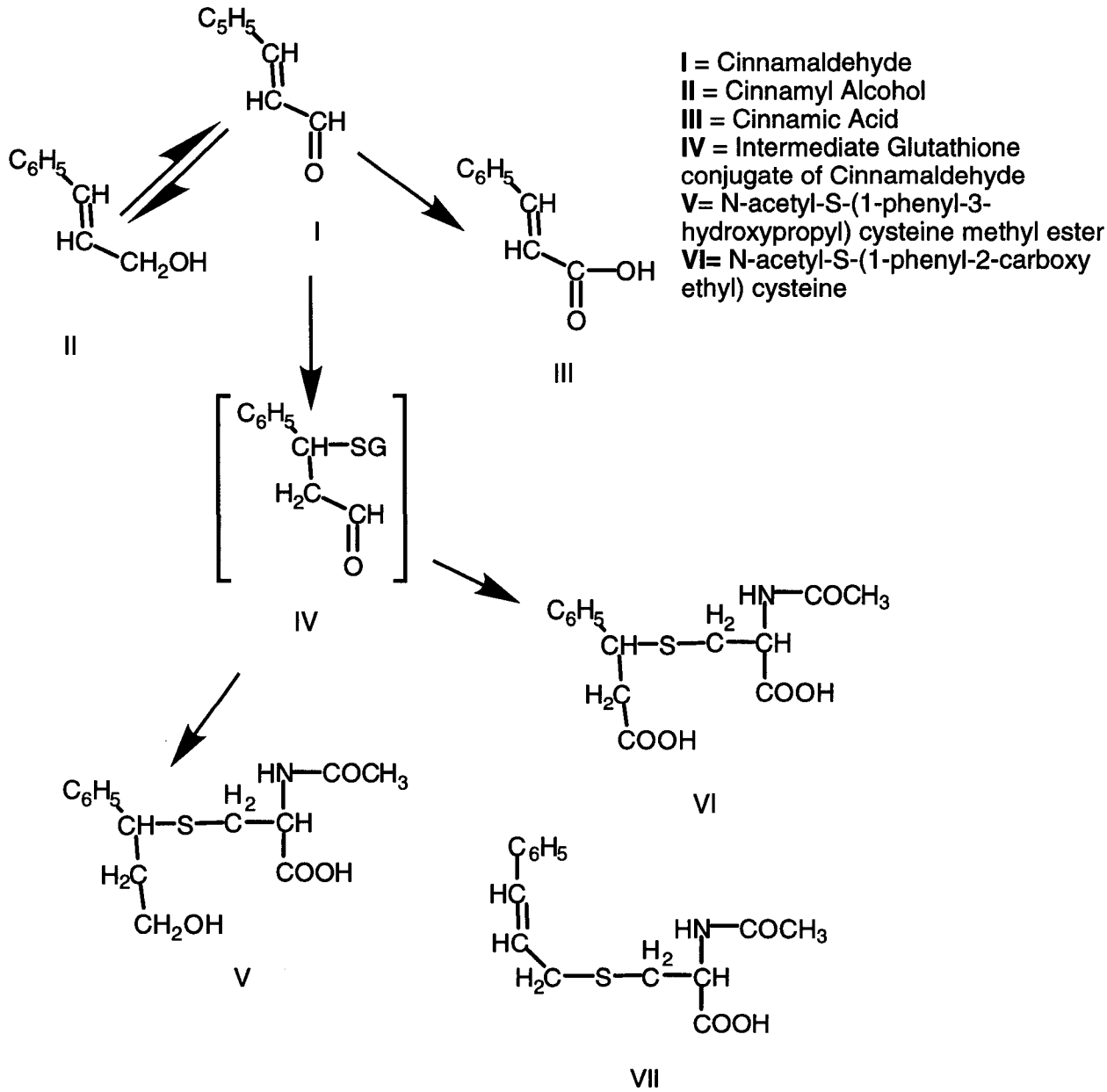
The absorption, distribution and excretion of Cinnamaldehyde labelled with Carbon-14 have been studied in male Fischer-344 rats following acute and subacute oral administration. Cinnamaldehyde labelled with 5-10 µCi/kg of Carbon-14 was administered by gavage at dose levels of 5, 50 and 500 mg/kg. For the acute studies, each rat was given a single, radioactive dose by gavage at one of the three dose levels. In the subacute studies, one dose of unlabelled Cinnamaldehyde was administered to groups of rats once a day for 7 days, followed by a single radioactive dose 24 hours after administration of the last unlabelled dose.

Following acute administration, Cinnamaldehyde was found to be excreted primarily in the urine, and within 72 hours after administration at the 50 and 500 mg/kg levels,

83.8 percent of the administered dose was excreted in the urine. Fecal excretion of radiolabel ranged from 5.1 percent of the dose for the 5 mg/kg dose to 10.5 percent for the 500 mg/kg dose.

FIGURE 2

METABOLIC PATHWAY OF CINNAMALDEHYDE IN THE RAT



Carbon-14 labelled Cinnamaldehyde was found to be distributed primarily to the gastrointestinal tract, liver and kidney in the acutely dosed rats, but after 24 hours

was reportedly cleared from the liver and kidney. An average of 5.2 percent of the administered radiolabel was found in the gastrointestinal tract after 24 hours at all dose levels. After 72 hours at the 50 and 500 mg/kg dose levels, the amount of radiolable found in the gastrointestinal tract was 0.19 percent and 0.39 percent of the administered dose respectively. Radiolabelled Cinnamaldehyde was distributed to the fat and was detectable in rats sacrificed 72 hours after dosing. Less than 0.1 percent of the administered dose at all three dose levels tested was distributed to the brain, heart, spleen, lung and testes. Estimated whole blood levels of Cinnamaldehyde averaged less than 0.1 percent of the administered dose after 24 hours at all dose levels tested.

Similar tissue distribution and excretion patterns were found following subacute dosing. A rapid clearance via the urine was observed 24 hours after administration, with an average of 81 percent of the administered radioactivity recovered in the urine, and an additional 5.9 percent recovered in the feces at all dose levels tested.

The administered radioactivity was found to be distributed primarily in the fat and gastrointestinal tract 24 hours after subacute administration at all dose levels. Liver accumulation accounted for less than 0.15 percent of the administered doses after 24 hours. Carbon-14 labelled Cinnamaldehyde was detectable in the fat at the 500 mg/kg dose level after three days. Less than 0.1 percent of the administered dose was observed in other tissues after 24 hours. The estimated level of radiolabelled Cinnamaldehyde was less than 0.1 percent of the administered dose in whole blood after 24 hours [63].

B. Human Data

There are no data available on the metabolism of Cinnamaldehyde in humans. Presumably, Cinnamaldehyde is oxidized to cinnamic acid which is excreted in the urine as benzoic and hippuric acids [16].

VII. BIOCHEMICAL TOXICOLOGY

A. Animal Data

Cinnamaldehyde has been found to be cytotoxic to L1210 mouse cells. The degree of cytotoxicity of Cinnamaldehyde was found to be proportional to the amount of the compound added to the cell culture medium. The ED_{50} value[9] of Cinnamaldehyde has been determined to be 4.8 μg/ml of culture solution.

The mechanism by which Cinnamaldehyde inhibits L1210 mouse cell growth was examined by studying the effect of Cinnamaldehyde on RNA, DNA and protein synthesis as well as its effect on glycolysis. Cinnamaldehyde at concentrations ranging from 0 to 50 μg/ml was added to cultures of L1210 cells at various intervals, and the resulting concentrations of glucose and lactate in the culture solution were determined enzymatically. The addition of Cinnamaldehyde to the culture media was found to have only a slight effect on glycolysis by L1210 cells (see Figure 4).

The effect of Cinnamaldehyde on RNA, DNA and protein synthesis was determined by measuring L1210 cell incorporation of tritiated Uridine, tritiated Thymidine, and tritiated Leucine at various time intervals. Among the labelled isotopes tested, the incorporation of tritiated Leucine was inhibited most strongly, indicating a preferential inhibition of Cinnamaldehyde on protein (see Figure 5). The toxic effect of Cinnamaldehyde on protein synthesis could be removed by transferring the cells to a Cinnamaldehyde-free medium, suggesting that Cinnamaldehyde did not cause irreversible cellular damage.

Cinnamaldehyde was subsequently found to inhibit the growth of L1210 cells by blocking protein synthesis through a direct interaction with sulfhydryl-containing amino acids. Sonicates were prepared from suspensions of L1210 cells inhibited by Cinnamaldehyde and analyzed for their sulfhydryl content. Cinnamaldehyde was found to reduce the sulfhydryl content of the sonicates in a dose-dependent manner (see Figure 6), suggesting a direct chemical interaction between Cinnamaldehyde and the sulfhydryl groups of the L1210 cell components.

This direct interaction was confirmed by the results of experiments in which Cysteine or Glutathione was allowed to react with various concentrations of Cinnamaldehyde. Glutathione, which was added to the reaction mixture as an additional source of sulfhydryl groups, showed minimal reaction with Cinnamaldehyde based on the concentration of residual sulfhydryl groups,
while Cinnamaldehyde was found to react directly with Cysteine (see Figure 7) [44].

The catecholamine-releasing effect of Cinnamaldehyde has been studied in male and female Mongrel dogs following intravenous and intraduodenal administration of 20 mg/kg of Cinnamaldehyde. It was observed that the total catecholamine concentration

reached a maximal level two minutes after intravenous administration, before returning to baseline, after approximately twenty minutes. No effect on blood pressure was observed. Extraction of the catecholamines from the samples and analysis of content revealed that the increased portion of catecholamines was epinephrine. Similarly, intraduodenal administration of 50 mg/kg of Cinnamaldehyde caused a dose-dependent increase in catecholamine concentration. Epinephrine accounted for nearly all of the increase in catecholamine, no significant change in norepinephrine concentration was observed.

Ganglion blocking has not been found to affect the catecholamine releasing property of Cinnamaldehyde. The influence of ganglion blocking on the catecholamine releasing effect of Cinnamaldehyde was determined by monitoring blood pressure during the co-administration of ganglion blocking agents (hexamethonium and atropine) and Cinnamaldehyde intravenously. In addition, the influence of the adrenals on the catecholamine releasing effect of Cinnamaldehyde was investigated. After surgically blocking adrenal circulation, Cinnamaldehyde was administered intraduodenally. The effect of

FIGURE 4[10]

EFFECT OF CINNAMALDEHYDE ON THE GLYCOLYSIS OF L1210 CELLS

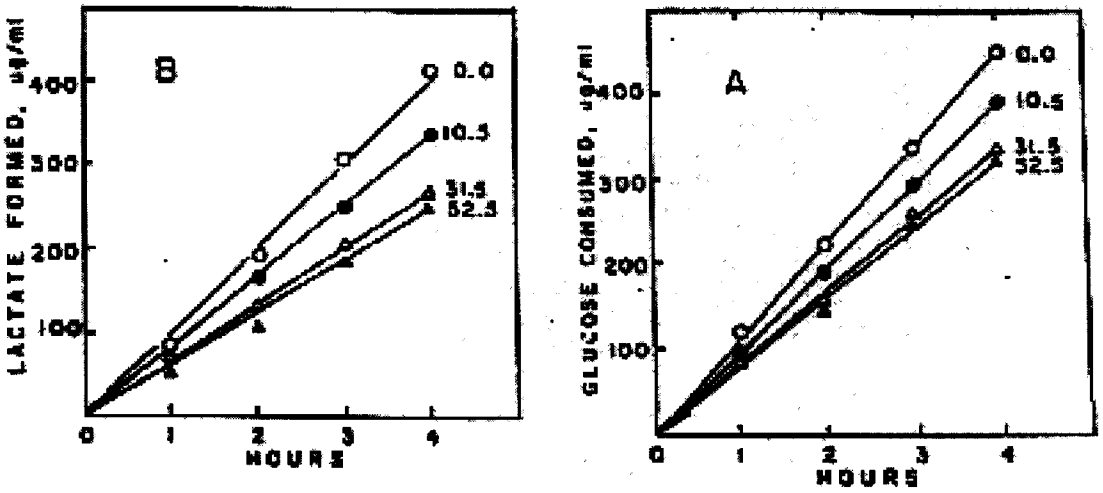


FIGURE 5[11]

MODE OF INHIBITION OF CINNAMALDEHYDE ON GLYCOLYSIS AND MACROMOLECULE BIOSYNTHESIS OF L1210 CELLS

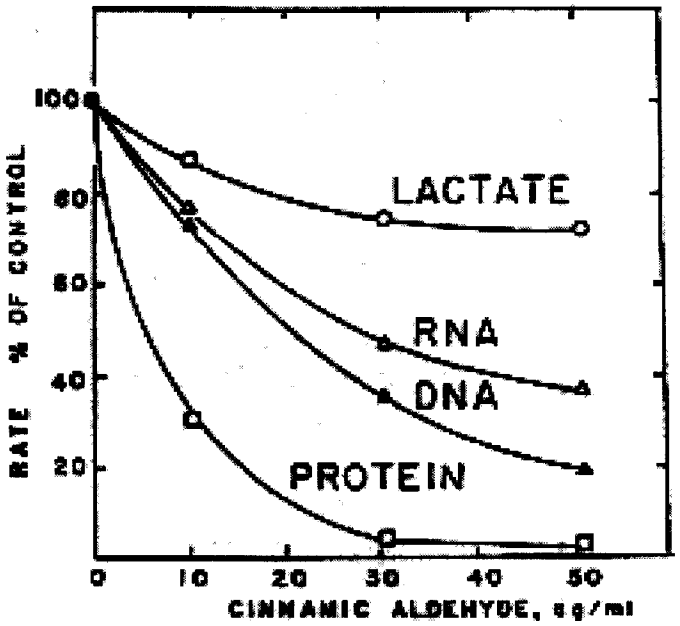


FIGURE 6

SULFHYDRYL CONTENT OF SONICATES PREPARED FROM SUSPENSIONS OF L1210 CELLS INHIBITED BY CINNAMALDEHYDE

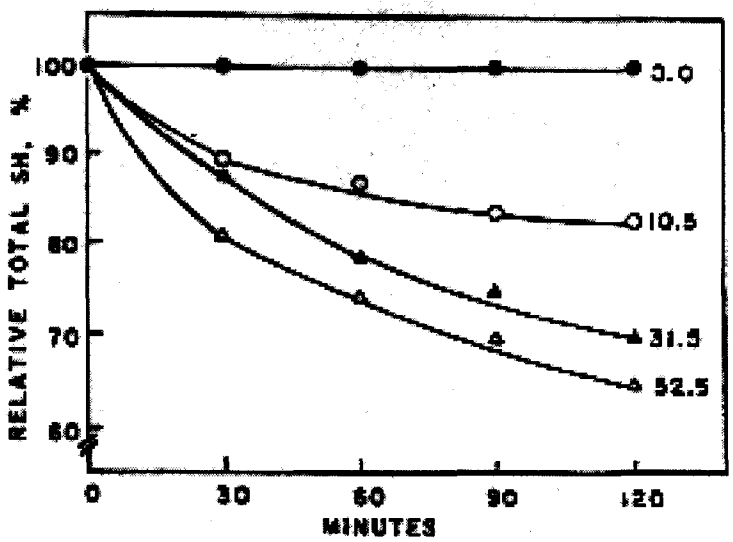
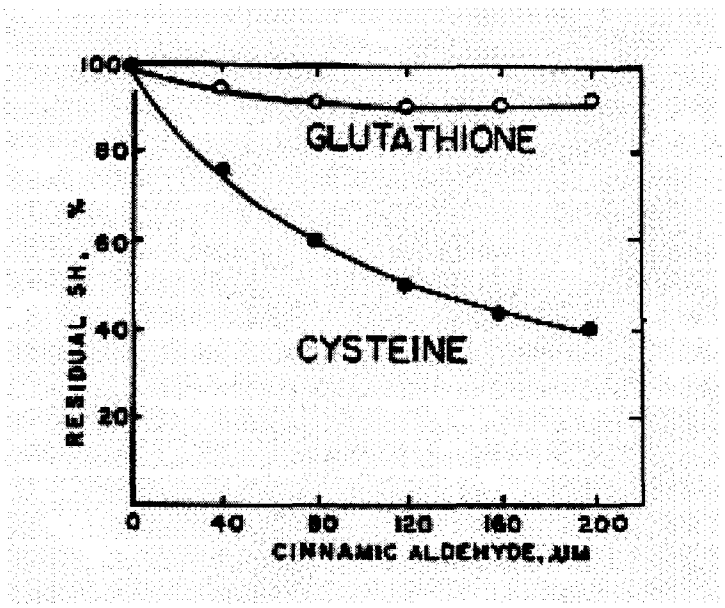


FIGURE 7

REACTION BETWEEN CINNAMALDEHYDE AND GLUTATHIONE IN BUFFER SOLUTION



Cinnamaldehyde was observed to disappear significantly after ligation of the adrenals, and the basal catecholamine level dropped to approximately half the baseline level before ligation. Based on this observation, it is believed that plasma catecholamines released following systemic administration of Cinnamaldehyde originate predominately from the adrenals. The finding that the catecholamine releasing effect of Cinnamaldehyde was not influenced by ganglion blocking indicates that this compound increases plasma catecholamine concentration through a mechanism independent of an increase in androgenic nervous activity [19].

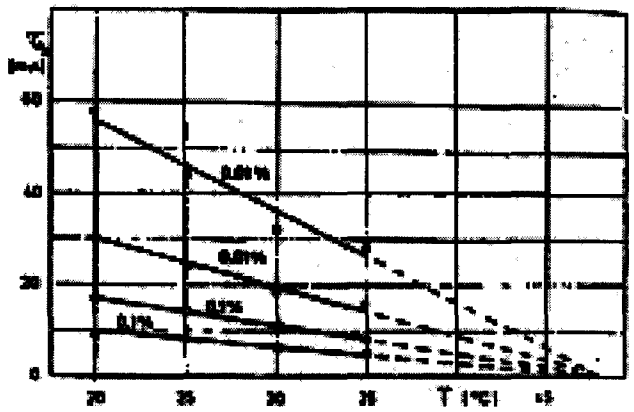
The kinetics of impulse blocking by Cinnamaldehyde in frog sciatic nerve have been tested at various temperatures and Cinnamaldehyde concentrations. The frog sciatic nerves taken from male specimens of *Rana temporaria* were ligatured to prevent inactivation by sodium before immersion in buffer solution. Varying concentrations of Cinnamaldehyde (0.01-0.10%) were introduced into a stimulation chamber[12]. Stimulating square pulses were delivered by a generator through a pulse separation unit and full size nervograms were obtained in order to assess the maximal action potential.

Cinnamaldehyde was found to decrease the amplitude of the nervogram in frog nerve up to complete blockage of the action potential, the rate of this effect depended on temperature and Cinnamaldehyde concentration. It was found that nerve excitement could be restored almost completely upon immersion in buffer solution without Cinnamaldehyde, so that the effect of a second treatment on the same nerve could be studied. This second blocking time was found to be shorter than the initial blocking at the same temperature and Cinnamaldehyde concentration (see Figure 8).

The impulse blocking rate of Cinnamaldehyde has been compared to that of other aldehydes. The following order indicates the relative speed of impulse blocking at the same temperature and concentration: Crotonaldehyde> Cinnamaldehyde > Butyraldehyde > Formaldehyde > Glutaraldehyde. Cinnamaldehyde was the only aldehyde tested that demonstrated a reversible blocking effect on nerve impulses [39].

FIGURE 8

IMPULSE BLOCKING EFFECT OF CINNAMALDEHYDE



The blocking time of nerve impulses as a function of temperature at two Cinnamaldehyde concentrations at a first treatment (o) and a second one (o) following recovery

B. Human Data

Cinnamaldehyde has been reported to have anti-platelet aggregating and vasodilatory action *in vitro* . Thromboxane A_2 (TxA_2), an Arachidonic Acid metabolite which is produced in platelets, is known to be a potent pro-aggregatory agent, and therefore the alteration of TxA_2 synthesis is believed to affect platelet aggregation.

In order to test the effect of Cinnamaldehyde on TxA_2 formation, platelet rich human plasma was incubated with various concentrations of Cinnamaldehyde and then stimulated with the aggregant, collagen. Cinnamaldehyde was observed to inhibit collagen-induced platelet aggregation in a dose dependent manner. A prolongation of the lagtime before the initiation of collagen-induced platelet aggregation was observed by the addition of increasing doses of Cinnamaldehyde. At a dose of 750 μM, Cinnamaldehyde almost completely suppressed collagen-induced platelet aggregation.

The effect of Cinnamaldehyde on a preparation of washed human platelets (5×10^5 μl) was examined. Again, the addition of Cinnamaldehyde was found to decrease collagen-induced platelet aggregation in a dose-dependent manner and nearly complete suppression was observed when platelets were pretreated with 300 μM Cinnamaldehyde.

In order to examine the effect of Cinnamaldehyde on Arachidonic Acid (AA) metabolism in human platelets, washed platelets were stimulated with collagen in the presence of Cinnamaldehyde and the concentration of AA-derived metabolites was measured. The addition of Cinnamaldehyde was found to dose dependent decrease the formation of Thromboxane B_2 (TxB_2), 12-Hydroxyheptadecatrienoic acid (HHT), and 12-hydroxy-eicosatetrgenoic acid (12-HETE)[13]. In addition, a positive correlation between reduced platelet aggregation and decreased TxB_2 formation in Cinnamaldehyde pretreated platelets was observed. The addition of Cinnamaldehyde at concentrations up to 300 μM to washed human platelets prelabelled with [^{14}C]-Arachidonic Acid (AA) had no significant effect on the conversion of [^{14}C]-AA to either [^{14}C]-TxB_2, [^{14}C]-HETE or [^{14}C]-HHT, indicating that Cinnamaldehyde does not affect the metabolism of Arachidonic Acid by either the cyclooxygenase or the lipoxygenase pathways. The action of Cinnamaldehyde was therefore believed to be proximal to the cyclooxygenase and lipoxygenase level.

In order to assess this possibility, the effect of Cinnamaldehyde on the collagen-stimulated release and metabolism of [^{14}C]-AA from washed human platelets was examined. The addition of Cinnamaldehyde was found to cause a dose-dependent decrease in collagen-induced prelabelled platelet aggregation as well as a dose-dependent reduction in the percentage of [^{14}C]-AA released, and the percentage of [^{14}C]-TxB_2 formed, from prelabelled platelets. In addition, there was a positive correlation between decreased platelet aggregation and reduced release of [^{14}C]-AA, as well as a positive correlation between decreased platelet aggregation and reduced formation of TxB_2. A positive correlation was also noted between the decreased release of [^{14}C]-AA and reduced formation of [^{14}C]-TxB_2.

These results indicate that the reduced production of Thromboxane B_2 (TxB_2) in Cinnamaldehyde pretreated platelets may most likely be a result of impaired Arachidonic Acid liberation from platelet membrane phospholipids, and not a result of the inhibition of AA metabolism via the cyclooxygenase pathway [66].

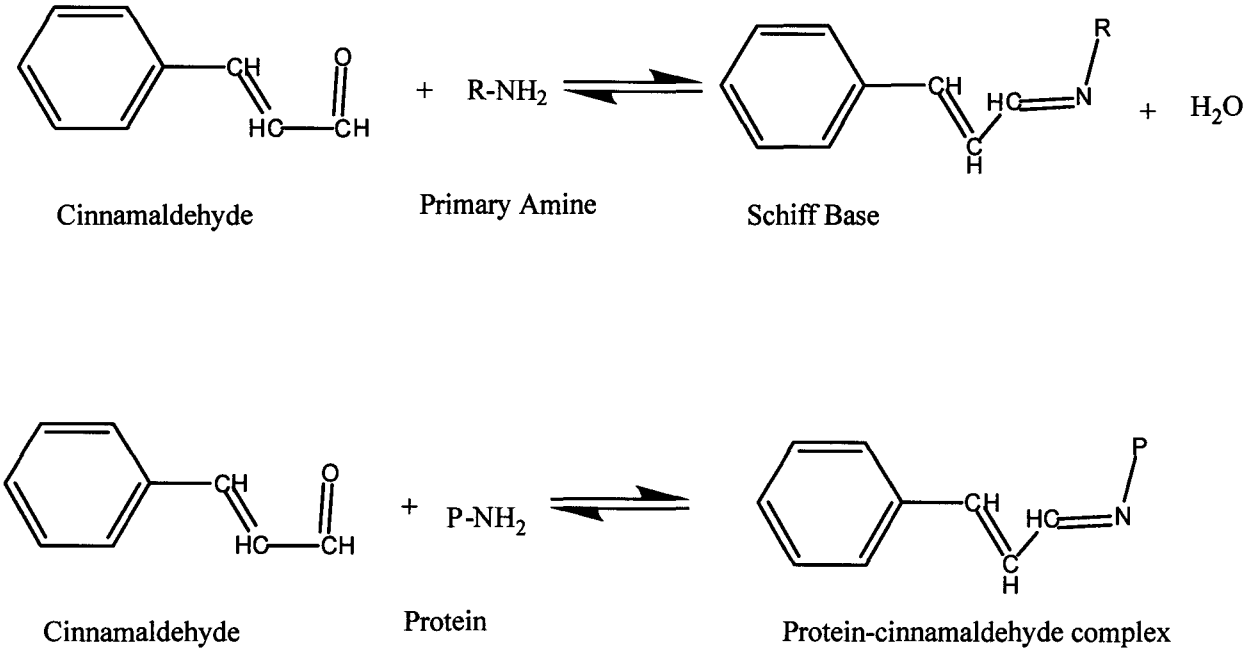
Several studies have been conducted to investigate the mechanism by which Cinnamaldehyde causes skin sensitization. It is generally agreed that Cinnamaldehyde, a low molecular weight substance, cannot induce contact allergy in the skin unless it is bound to a protein. However, it is uncertain which proteins react with

Cinnamaldehyde, and it is unclear which reacting groups are involved.

It has been proposed that skin sensitization mechanism involves the Schiff base ligands on the protein side chains which initiate the allergenic response (see Figure 9). In order to investigate this, the reactivity of a series of "-alkyl substituted Cinnamaldehydes with amines was compared. When Cinnamaldehyde was allowed to react with Cyclohexylamine, the reaction was rapid, yielding the expected Schiff base. Alpha-Methyl, "-Amyl and "-Hexyl Cinnamaldehyde, which have been shown to be non-sensitizers, did not react with Cyclohexylamine. The chemical inactivity of "-alkyl substituted Cinnamaldehydes relative to that of Cinnamaldehyde may explain why these Cinnamaldehyde derivatives are non-sensitizers [37].

FIGURE 9

REACTION OF CINNAMALDEHYDE WITH PRIMARY AMINES AND PROTEINS



A more recent study supports the theory that the formation of a Cinnamaldehyde-protein conjugate in the skin is via Cinnamaldehyde binding sites on the protein that appear to be predominantly the thiol groups of cysteine residues.

Reactions between Cinnamaldehyde and various nucleophiles have been carried out using model compounds containing thiol nucleophiles such as Cysteine, N-Acetylcysteine and Thioethanol, as well as model compounds containing amine nucleophiles including Lysine, Alanine, Glycine, Propylamine and Imidazole. The reactions were performed at pHs ranging from 7.4 to 10.5 with the total nucleophilic concentration in excess of the Cinnamaldehyde concentration. By monitoring the concentration of Cinnamaldehyde spectrophotometrically and by high performance liquid chromatography (HPLC), the reactions were determined to follow pseudo-first-order kinetics. The observed pseudo-first-order rate constants (K_{obs}) were found to follow the rate expression:

$$K_{obs} = k_o + k_r \text{ (nucleophile)}$$

where k_o represents the rate constant at zero nucleophile concentration and k_r the nucleophilic attack of nucleophiles respectively on Cinnamaldehyde.
Considerably higher second order rate constants were found for the reaction of Cinnamaldehyde with thiol nucleophiles than for reaction of Cinnamaldehyde with amine nucleophiles, indicating that the free thiol groups of Cysteine residues are the sites to which the Cinnamaldehyde molecule is primarily bound (see Table 3.1) [74].

The passage of Cinnamaldehyde through human skin has been investigated by *in vitro* penetration studies using full thickness human skin. Abdominal skin samples were obtained and stripped of adipose tissue, yielding a skin membrane of epidermis and dermis of approximately 2 mm. Cinnamaldehyde, at a concentration of 200 mg/ml, was added to the epidermal side of the skin which had been enclosed by a glass diffusion cell. Samples taken from the receptor phase were analyzed by HPLC after precipitation of the protein. This analysis revealed that Cinnamyl alcohol and Cinnamic Acid were found in the receptor phase at a higher concentration than Cinnamaldehyde (see Figure 10). Only a small amount of unchanged Cinnamaldehyde was detected in the receptor phase, suggesting a loss of cinnamaldehyde either by degradation in the receptor phase or by an enzyme/non enzyme mediated conversion during diffusion of the Cinnamaldehyde through the skin.

TABLE 3.1

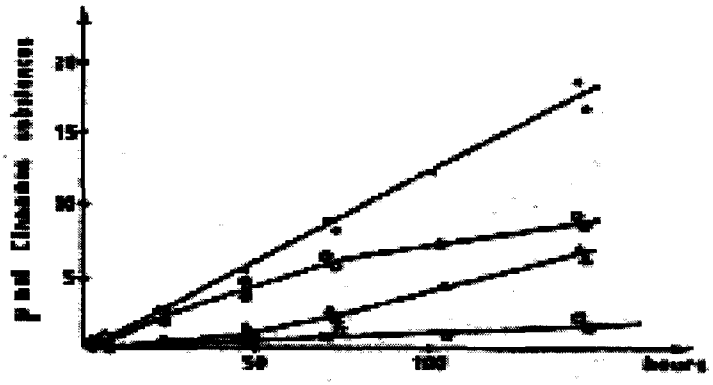
SECOND-ORDER RATE CONSTANTS (Kr) FOR FORMATION OF CONJUGATES BETWEEN DIFFERENT NUCLEOPHILES AND CINNAMALDEHYDE

pH	Nucleophile	k_r (M^{-1} min^{-1})
6.4	cysteine	13
7.4	cysteine	107
8.3	cysteine	440
6.4	N-acetyl cysteine	0.12
7.4	N-acetyl cysteine	1.0
8.2	N-acetyl cysteine	2.0
7.4	thioethanol	-1.7
7.4	lysine	n.d.
7.4	glycine	5.4×10^{-4}
8.6	glycine	7.2×10^{-3}
7.4	alanine	n.d.
8.8	alanine	1.4×10^{-3}
10.5	propylamine	7.2×10^{-4}
7.4	phenol	n.d.
7.4	imidazole	n.d.
10.5	imidazole	n.d.

n.d. = no detectable reaction

FIGURE 10

IN VITRO PERCUTANEOUS PENETRATION OF CINNAMALDEHYDE



• = µmol Cinnamon (alcohol + aldehyde + acid)

o = µmol Cinnamyl Alcohol

a = µmol Cinnamic Acid

⊔ = µmol Cinnamaldehyde

The permeability coefficient, which represents the penetration rate of Cinnamaldehyde, was calculated at
3.8×10^{-5} cm/hr. The amount of Cinnamaldehyde transformation in the skin was estimated by the following equation:

$$q_r = (k_o - Q) \frac{i - e^{-Kt}}{k}$$

where q_r represents the amount of Cinnamic substances in the receptor medium, k_o represents the steady-state flux of the Cinnamic substances through the barrier, Q represents the steady state transformation of Cinnamaldehyde in the skin and K represents the first order rate constant for the transformation of Cinnamaldehyde in the receptor medium.

A plot of total appearance of Cinnamic substances in the receptor phase versus $(i - e^{-Kt})/k$ was found to yield a straight line, with the slope (1.2×10^{-7} mol h^{-1}) representing the steady-state transformation rate. These results indicate that approximately 90 percent of the Cinnamaldehyde applied to the epidermal side of the skin was transformed [75].

The mechanisms involved in the transformation of Cinnamaldehyde in human skin have been studied using Bovine Serum Albumin (BSA) as a model. Cinnamaldehyde (3×10^{-5}M) was incubated with and without BSA and in both cases the Cinnamaldehyde was degraded to Cinnamic Acid and Cinnamyl Alcohol. However, in the presence of BSA, the degradation rate was approximately four times higher ($K_{obs} = 0.16\ h^{-1}$ in the presence of BSA, versus $4.1 \times 10^{-2}\ h^{-1}$ in the absence of BSA), indicating that the protein contributes to the overall disappearance of the Cinnamaldehyde [30].

From analysis of Cinnamaldehyde-BSA conjugates formed following incubation of the two compounds, it was determined that the nucleophilic groups in the proteins to which the Cinnamaldehyde moieties were bound appeared to be primarily thiol groups, and the number of thiol groups
corresponded closely to the number of Cinnamaldehyde groups introduced [74].

VIII. STRUCTURE/ACTIVITY CONSIDERATIONS

Cinnamaldehyde has been found to inhibit the growth of L1210 mouse cells with an ED_{50} value of 4.8 μg/ml. However, Cinnamic Acid and Cinnamic Alcohol, both having molecular structures similar to Cinnamaldehyde, did not significantly affect the growth of L1210 cells. This result indicates that the inhibitory activity of Cinnamaldehyde may reside primarily in the aldehyde portion of the molecule (see Table 4) [44].

TABLE 4

ED_{50} VALUES OF CINNAMIC ALDEHYDE, CINNAMIC ACID AND CINNAMIC ALCOHOL

Compound	**R[a]**	**ED_{50} (fg/ml)**
Cinnamaldehyde	CHO	4.8
Cinnamic Acid	COOH	>100
Cinnamic Alcohol	CH_2OH	76

[a]C_6H_5-CH=CH-R

Substitutions in the alpha-Carbon position of Cinnamaldehyde have been found to influence the mutagenicity of this compound. Results from a study that compared the mutagenicity of Cinnamaldehyde to several alpha-carbon substituted derivatives in *Salmonella typhimurium* strain TA100 indicate that Cinnamaldehyde is non-mutagenic to this strain of *Salmonella* in the presence and absence of metabolic activation. However, the halo derivatives of Cinnamaldehyde, alpha-Chlorocinnamaldehyde and alpha-Bromocinnamaldehyde were found to be strong, direct acting mutagens [16]. Presumably, the alpha-Chloro and the alpha-Bromo substituents cause an increase in electrophilicity of the beta-Carbon atom, thus promoting nucleophilic addition reactions at the double carbon bond. The electrophilicity of the beta-Carbon atom has been shown to be a critical parameter for reactivity with cellular nucleophiles, including DNA[53].

Although there are no studies reported in the literature associating Cinnamaldehyde with carcinogenic effects in animals or humans, there are two related compounds that have been reported to induce tumors in experimental animals. 3,4,5-Trimethoxy Cinnamaldehyde has been found to induce tumors in rats following intraperitoneal injection. In addition, Cinnamyl Anthanilate has been found to cause tumors in both rats and mice by dietary

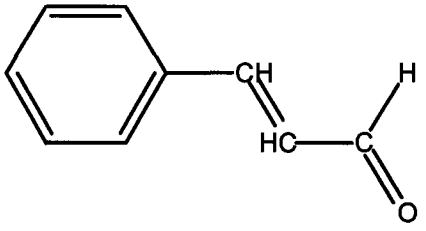
administration at 15,000 or 30,000 ppm [3]. Because Anthranilic Acid was not found to be carcinogenic when tested in mice or rats it is believed that the Cinnamyl moiety may play a role in the carcinogenicity of Cinnamyl Anthranilate [3] (see Figure 11).

As an aldehyde, Cinnamaldehyde is a potential alkylating agent. Through its reaction with amino groups in cellular macromolecules, this compound forms Schiff base intermediates. Cinnamaldehyde is also a potential alkylating agent via epoxidation of the double bond.

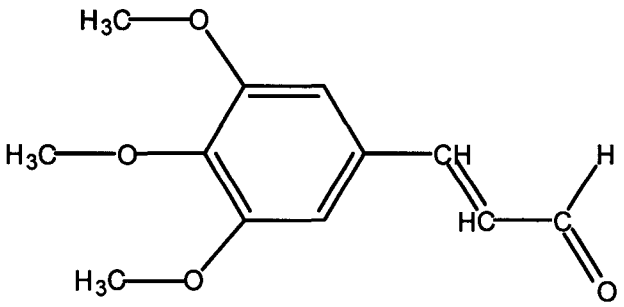
FIGURE 11

CINNAMALDEHYDE AND STRUCTURALLY RELATED COMPOUNDS

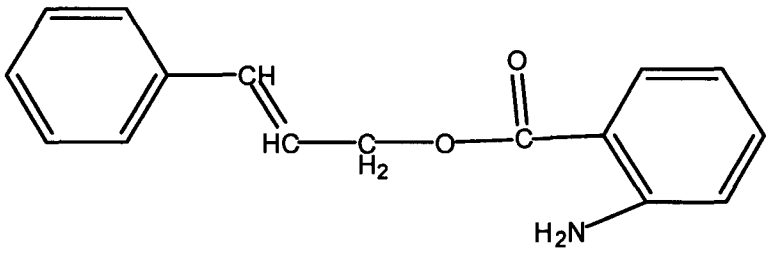
Cinnamaldehyde



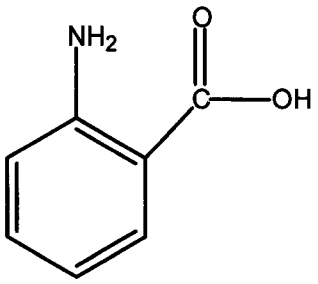
3,4,5-Trimethoxycinnamaldehyde



Cinnamyl Anthranilate



Anthranilic Acid



ON-LINE DATABASES SEARCHED

MEDLARS

Chemline	
Chemlist	
RTECS	
Hazardous Substance Databank (HSDB)	
HZDB	
Toxline	1981-Present
Toxline 65	1965-Present
Toxlit	1981-Present
Toxlit 65	1965-1980

DIALOG

Agricola	1970-Present
Aquatic Science Abstracts	1978-Present
Biosis Previews	1969-Present
CA Search	1967-Present
Chemical Regulations and Guidelines system	November 1989
CHRIS USDA	September 1989
Compendex Plus	1970-Present
EMBASE	1974-Present
Environmental Bibliography	1974-Present
FDC Reports	1987-Present
Federal Register	1979-Present
Foods Adlibra	1974-Present
FSTA	1969-Present
Life Sciences Collection	1978-Present
MEDLINE	1966- Present
NTIS	1964-Present
Occupational Safety and Health	1973-Present
Pharmaceutical News INdex	1974-Present
Pollution Abstracts	1970-Present
PTS PROMPT	1972-Present
Trade and Industry	1983-Present
Trade and Industry Index	1981-Present

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[1] The ID_{50} value represents the dose required to inhibit cell growth by 50%.

[2] Vitamin A acetate was added to the diet in order to amplify the presentation of immunogenic agents.

[3] A statistically significant increase in the absolute number of foci and in the enhancement ratio is indicated by ** (P#.01) or * (P#.05)

[4] The D_{20} value represents the dose (mg/ml) at which structural abberations, including gaps, were detected in 20% of the metaphase chromosomes observed.

[5] The TR value indicates the frequency of cells with exchange type aberrations per unit dose (mg/ml).

[6] The least effective concentration (LEC) represents the lowest dose to give a statistically significant increase (P# 0.05) in aberrations or a 20% increase in SCEs.

[7] The reproductive index is a measurement of the number of females that produced viable litters, divided by the number of proven pregnant females (multiplied by 100).

[8] The Optimal Teratogenic Dose (OTD) is defined as the concentration that induces a maximum teratogenic effect beyond the limits of the embryonic LD_{50}.

[9] The ED_{50} value represents the concentration of test compound that inhibits cell growth by 50%.

[10] Numbers next to the plotted lines indicate Cinnamaldehyde concentrations (Fg/ml) in cell culture medium.

[11] Numbers indicate amount of Cinnamaldehyde added (Fg/ml)

[12] The stimulation chamber has 7 compartments formed by plexiglass septa sealed with vasoline. In the first 2 chambers from both ends, there are stimulating and recording electrodes. The entire chamber is thermostat controlled and a Cu-constantan thermocouple is located in the central compartment.

[13] TxB_2 and HHT are metabolites of Arachidonic Acid produced via the cyclooxygenase pathway. The Arachidonic Acid metabolite 12-HETE is produced via the lipoxygenase pathway.