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Notification of Claim for General Recognition of Safety of Allyl Isothiocyanate in a Food Shelf Life Extension and Anti-spoilage System

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ERIC F. GREENBERG, P.C.
A professional corporation

August 10, 2005

RECD AUG 11 2005

Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food And Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Re: NOTIFICATION OF CLAIM FOR GENERAL RECOGNITION OF SAFETY OF ALLYL ISOTHIOCYANATE IN A FOOD SHELF LIFE EXTENSION AND ANTI-SPOILAGE SYSTEM

To the FDA:

This letter and its attachments contains the notification, pursuant to the Federal Food, Drug and Cosmetic Act and FDA's regulations including proposed 21 CFR 170.36 published on April 17, 1997 (62 FR 18937), by Mitsubishi-Kagaku Foods Corporation, 3-9, Ginza 1-chome, Chuo-ku, Tokyo, Japan, c/o attorney Eric F. Greenberg, Eric F. Greenberg, P.C., 3500 Three First National Plaza, Chicago, Illinois 60602[1] ("Mitsubishi"), of the General Recognition of Safety of Allyl Isothiocyanate ("AIT") for use as a shelf-life extension agent or anti-spoilage agent in a food container system for use in the home, in foodservice establishments, and in the farm field.

[1] Attachment 1 contains Mitsubishi's authorization of representation by undersigned counsel.

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Under the described use, the AIT would be incorporated into food containers in order to deliver 30-50 parts per million of AIT to food held in the container.

As set forth more fully below and in the attachments to this document, Mitsubishi believes the intended use of AIT, Chemical Abstracts Service number 57-06-7, is GRAS, and therefore exempt from the premarket approval requirements of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. Sec 301 et seq., based on scientific procedures within the meaning of 21 U.S.C. Sec. 321(s) and FDA's implementing regulations in 21 CFR Sec. 170.30, and including FDA's proposed regulation 21 CFR 170.36 published on April 17, 1997 (62 FR 18937). As required by FDA regulations, the scientific evidence of safety relied upon and summarized here is generally known and accepted by qualified experts in the appropriate scientific and technical fields, 21 CFR Sec. 170.30(a), and is available for FDA review.

The use described here involves the same substance as was the subject of GRAS Notice 133, submitted by Carex, Inc.[2] and the subject of a favorable FDA letter dated January 5, 2004. This GRAS

[2] The assets of Carex, Inc. are now owned by Mitsubishi-Kagaku Foods Corporation.

Notice, unlike the limited scope of GRAS Notice 133, contemplates the potential for exposure across the entire diet that is packaged in polymers. This submission relates to a similar material used in a similar way for a similar purpose, but provides a more complete safety analysis and more thorough analysis of the existing exposures to AIT than those in GRAS Notice 133.

I. Claim of Exemption

a. Name and address of the notifier.

Mitsubishi-Kagaku Foods Corporation
c/o Eric F. Greenberg
Eric F. Greenberg, P.C.
3500 Three First National Plaza
Chicago, IL 60602

b. Common or usual name of the notified substance.

Allyl isothiocyanate (AIT)
Volatile oil of mustard
Mustard oil
Oil of black mustard
Oleum sinapis
Senfoel
Allyl mustard oil
Allyl isothiocyanate (natural)
Allyl thioisocyanate (natural)

c. Conditions of use (foods, levels, purposes).

In the applications encompassed by this GRAS Notice, a variety of delivery technologies, each delivering similar levels of AIT into the air, may be used. For example, a "patch" containing AIT at a specified level and of a specified size and shape will be affixed to the flexible polymer, or a flexible film will be prepared containing AIT at a specified level and of a specified size. The AIT will be contained within microcapsules that will dissolve when the humidity reaches 70% or above, thereby releasing the AIT into the ambient air. The patch is made of conventional polymers including polyethylene with the AIT master batch in it. The patch will have an adhesive on the back for application into the bag, or be heat-sealed.

Neither the material used to create the microcapsules, nor the adhesive, are reasonably expected to become components of the food in the container under the intended conditions of use.

When used as described in this Notice, AIT meeting appropriate purity specifications is used to provide freshness extension for foods in food storage packaging for use in the home; in foodservice establishments (restaurants, cafeterias and grocery stores); and in farm fields to pack raw agricultural commodities. The food preservation system releases AIT into airspace found in storage

bags and other containers, and it preserves the freshness of the food by exerting an antimicrobial effect on spoilage bacteria in or on the food within the bag or other container.

The AIT patch activates in the presence of moisture so that AIT is released when the relative humidity increases, i.e., when food is added to the bag or other container. The microencapsulated AIT remains shelf-stable or dormant until the food is placed in the bag or container. When the relative humidity exceeds 70%, AIT will be released.

The notifier has determined that freshness extension occurs at 30-50 ppm, therefore the release rate of the patch will be 30-50 ppm. If the food in the bag absorbs the AIT in the atmosphere of the bag or other container or the AIT escapes through the packaging material, then the patch will release more AIT in order to maintain a steady state.

AIT's ability to retard the growth of a wide range of common bacteria, fungi, molds and yeast has been well established and was summarized in GRAS Notice 133, p.5. As recognized in FDA's letter summarizing its review of GRAS Notice 133, exposure of food to AIT in this manner, that is, in an aerobic environment, is not expected to

mask spoilage or mask the presence of anaerobic pathogens. As FDA noted in its letter to Carex in response to GRAS Notice 133, "Because fully aerobic conditions are maintained under the intended conditions of use, Carex considers that its intended use of VOM will not increase the risk of outgrowth of anaerobic pathogens such as *C. botulinum*." Similarly, the proposed use does not encompass anaerobic conditions.

d. Basis of GRAS determination: Scientific procedures

AIT safety: Dietary exposure

This section provides an assessment of dietary exposure to AIT from background exposures and from the use of the Mitsubishi food preservation system. The data provided will demonstrate that the Notifier's use of AIT in a food preservation system will not significantly increase the overall exposure to AIT.

Background

As explained below, the US population is already exposed daily to at least an estimated 19.65 mg/person/day of AIT from naturally

occurring food, food flavoring uses, and other sources. The Mitsubishi use is expected to add only minimally to the dietary exposure to AIT, and the resulting cumulative levels are easily demonstrated to be both safe and generally recognized as such.

Potential dietary exposures to AIT from members of the Brassica family can be estimated based upon published reports of sinigrin levels in these food commodities. Sinigrin is a glycoside with an alkyl sidechain found in many common foods, including black or brown mustard (*Brassica nigra* or *Brassica juncea*), other members of the Brassica family such as cauliflower, broccoli, Brussels sprouts, cabbage, kale, and collards, and in foods such as watercress, papaya and horseradish. It is one of over 100 glucosinolates that have been found in food and is the only glucosinolate that can be metabolized to AIT, a volatile chemical that is responsible for the biting, nose-clearing characteristics of foods such as mustard and horseradish (Mithen et al., 2000).

Glucosinolates remain chemically stable in the cytoplasm of plant cells until the tissue is disrupted. Sinigrin is readily converted to AIT in aqueous solution and enzymatically though the action of the myrosinase (thioglucoside glycohydrolase EC 3:2:3:1).

Glucosinolates are hydrolyzed to equimolar concentrations of glucose, aglycone and sulphate and the unstable aglycone from sinigrin subsequently reacts to form AIT. The extent of conversion is highly variable and depends upon factors such as pH, temperature and the presence of myrosinase.

Estimation of AIT Exposure from the Consumption of Broccoli, Cauliflower, Brussels sprouts and Cabbage

Many analyses have been reported for sinigrin-containing commodities that constitute important components of the diet of individuals in the United States. In many cases, these analyses include both the raw and cooked form of the commodity. Concentrations are typically less after cooking and, in cases where sinigrin levels were measured after cooking, that data was incorporated into the dietary intake estimates.

Dietary exposure estimates of AIT were based upon published values for sinigrin in various commodities. These estimates are based on the assumption that there was complete conversion of

sinigrin to AIT in the food, in the gastrointestinal tract or after absorption of foodstuffs containing sinigrin.

Table 1 shows the reported levels of sinigrin in broccoli, cauliflower, cabbage and Brussels sprouts in studies from the published literature. Levels of sinigrin were often reported as means in the published literature and those means were weighted by the number of samples analyzed to determine means for the raw and cooked forms of each commodity. Mean levels of sinigrin were then converted to AIT equivalents by converting sinigrin to AIT on a molar basis. This approach allowed the estimation of mean sinigrin and potential AIT levels in cooked and uncooked cabbage, broccoli, Brussels sprouts and cauliflower.

Estimates of consumption for these sinigrin-containing commodities were obtained from the US Department of Agriculture's Continuing Survey of Food Intakes by Individuals (CSFII). Data from the CSFII 1994-1996 survey were the basis for the consumption estimates.[3]

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[3] Approximately 16,000 individuals participated in the survey over a period of three years. Using a stratified sampling technique, individuals of all ages living in households in all 50 states and Washington DC were surveyed. Individuals were asked to recall their dietary intake for two nonconsecutive days based upon 24-hour recall data. Appropriate demographic data were also collected during the survey. Average daily intakes for commodities were determined and were

AIT equivalents derived from sinigrin concentrations are shown for the four commodities (cabbage, Brussels sprouts, cauliflower and broccoli) that are expected to have the largest dietary contributions to AIT exposure. They were derived as follows.

Sinigrin concentrations were different for raw and cooked commodities and concentrations for the raw and cooked forms the commodities were then multiplied by the amounts consumed of the raw and cooked dietary items. AIT equivalents for cooked Brussels sprouts were multiplied by the mean total consumption for this commodity because the CSFII database found that there was no reported consumption of uncooked Brussels sprouts. Exposures from the cooked and uncooked forms of the commodity were then summed to produce a total estimated AIT intake for each commodity. The estimated intakes for AIT from cabbage, Brussels sprouts, cauliflower and broccoli were added together to give a total estimated

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expressed on a g/kg of body weight basis. Although food consumption was estimated on "per capita" and "consumer only" basis, for purposes of the estimation of consumption of AIT from Brassica species, mean consumption estimates were for consumers only. Geometric mean consumption estimates were derived from the Cumulative and Aggregate Risk Evaluation Software (CARES), version 1.1. Mean and distributional data for most of these commodities can also be found in the US EPA document entitled "CSFII Analysis of Food Intake Distributions", EPA/6000/R-03/029, March 2003.

consumption from these Brassica species for those who consume these items.

These estimated intakes are shown in the following table:

Table 1: US Dietary Intake of AIT from Four Brassica Species

Commodity	AIT Concentration (µg/kg food)	Geometric Mean Consumption of Commodity (g/kg bw)	Intake of AIT (µg/kg bw/day)
Cabbage	59.37 (uncooked) 19.25 (cooked)	0.048 (uncooked) 0.266 (cooked)	7.95
Brussels Sprouts	65.72 (cooked)	1.95 (cooked)	128.1
Cauliflower	31.29 (uncooked) 41.12 (cooked)	0.390 (uncooked) 1.033 (cooked)	54.95
Broccoli	6.54 (uncooked) 0.004 (cooked)	3.08 (uncooked) 0.005 (cooked)	3.07
Total			194.07

The four commodities shown in the above table resulted in an intake of 194.07 µg/kg bw/day of AIT. For a 60 kg person, this is equal to an intake of 11.84 mg/person/day of AIT.[4]

[4] Although no estimate of AIT intake from vegetables in the United States was found in the scientific literature, Stones *et al.*, (1984) reported that total glucosinolate intake from cruciferous vegetable intake in the UK was estimated to be approximately 50 mg/person with 18 mg/person being attributable to isocyanate-producing glucosinolates such as sinigrin. Fenwick and Healy (1983) estimated a total intake of 12-16 mg/person of

High consumers of Brussels sprouts or cauliflower, commodities with high sinigrin concentrations, may have much greater intakes of sinigrin than the estimated intakes for consumers with mean levels of intake. For example, the individual associated with the highest reported intake of Brussels sprouts in the CSFII survey (11.55 grams per kg bw, or 693 g/day of Brussels sprouts) would have an estimated intake of 88.8 mg/day of AIT. Estimated exposures for these four commodities are presented only for the geometric mean consumption and residue levels because it is considered very unlikely that a consumer will have extremely high intake of all four of these commodities.

Notifier believes its estimated exposures are scientifically valid and based on sound principles. Several comments are appropriate for a full understanding of the estimated dietary intake set forth above.

- First, the conversion of sinigrin in the diet to AIT is variable and has not been fully quantified. Human gastrointestinal flora *in vitro* has been shown to convert sinigrin to AIT with a 19% efficiency (Krul et al., 2002), but further

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glucosinolates for the Canadian population but did not describe the basis for their estimate.

conversion occurs after sinigrin is absorbed or in the more alkaline portions of the gastrointestinal tract. Rouzaud et al., (2004) found that 37% of sinigrin ingested in raw cabbage was excreted in the urine as AIT. Significant excretion of AIT also occurs via feces and exhaled air. Measurement of urinary excretion of AIT clearly underestimates the total conversion of sinigrin to AIT. In addition, significant conversion by myrosinase of sinigrin to AIT may occur *in situ* in food commodities resulting in the direct consumption of AIT (Yu et al., 2001).

- Second, processing and cooking of food may produce or degrade AIT (Engel et al., 2002) and only a small number of methods of cooking have been assessed in the studies used in this determination of AIT exposure.

- Third, other food sources of sinigrin probably exist, but were not incorporated into the assessment of background exposure. Sinigrin has also been found at relatively high levels in other members of the Brassica family such as kale, mustard

greens, collards and turnip greens and in members of the Allium family such as onion and garlic. Contributions from these commodities were not assessed here due to the small number of analyses reported in the scientific literature and the absence of studies showing the effects of cooking on sinigrin levels in these commodities.

- Fourth, there is also additional potential indirect dietary exposure to glucosinolates through meat and milk from animals fed forage Brassica or rapeseed meal (Fenwick and Heaney, 1983) but AIT intake from this source has not been quantified. The available data is insufficient to quantify this indirect source of exposure to AIT.

- Finally, sinigrin concentrations in Brassica commodities are influenced by factors such as the variety of commodity, method of cultivation, time of harvest and stresses such as insect damage or bruising during harvesting (Heaney and Fenwick, 1980). It is not known whether or not the Brassica commodities that were analyzed in the reports from

the open scientific literature are representative of the foods as they would be consumed in the US diet.

Estimation of AIT Intake from Mustard or Horseradish as Flavoring Agents

In addition to dietary intake of AIT from the consumption of Brassica species, there is further dietary intake of sinigrin, and hence AIT, from flavoring agents such as horseradish and mustard. These commodities are applied to food by consumers or added as flavoring agents to other foods that are subsequently sold to consumers.

Concentrations of sinigrin in mustard are highly variable, with brown and oriental mustards having the highest concentrations (Tsao et al., 2002). The maximum amount of sinigrin found in mustard bran is approximately 2.2% and the maximum amount of AIT present is 0.5%. Assuming that all sinigrin in mustard bran is converted to AIT, the maximum amount of AIT that people could be exposed to is 2.7% of the bran by weight (2.2% sinigrin, 0.5% AIT).

The Federation of American Societies of Experimental Biology (FASEB), acting through the Life Sciences Research Office, concluded in 1975 that average dietary intake of AIT from the use of

yellow and brown mustard as flavoring agents and condiments was 6 mg/person/day based upon a survey conducted by the National Research Council (FASEB, 1975). Sources of mustard in the diet that were quantified in the survey included use in condiments, relishes, fats and oils, nut, gravies and curries, sauces, baked goods, snack foods, processed vegetables and juices, and meat and fish products. Reports of the annual usage by the food industry in 1970, however, were noted by FASEB as suggestive of lower estimates of exposure to mustard from flavoring and condiment use than those found in the National Research Council survey.

If it is assumed that the FASEB estimate is accurate and still relevant to the US diet, an additional 6 mg/person/day of AIT may arise from the use of mustard as condiments and flavors in the US diet.

Mustard was not included in the CSFII survey of foods consumed in the United States and the 1975 estimates of mustard intake have not been revisited in this assessment of AIT dietary intake. No estimates of mustard consumption were available from the scientific literature or other sources and the data quantifying

levels of sinigrin or AIT in mustards commonly consumed in the United States are limited.

There is very limited data regarding the levels of sinigrin or AIT in horseradish. A single study from Eastern Europe is available which reports a total of 141.91 mg sinigrin/100 g of fresh horseradish (Hrncirik and Velisek 1997). Consumption of horseradish is low in the United States and horseradish would be expected to contribute little to dietary burden for AIT. The CSFII database found that the geometric mean consumption of horseradish for consumers of this commodity is 0.0168 µg/kg bw/day. This consumption estimate and the concentration of sinigrin in horseradish noted above would result in 5.67 µg/kg bw/day (0.340 mg/person) of AIT intake if it is assumed that there is complete conversion of sinigrin to AIT.

AIT Exposure from Currently Used Food Preservation Systems

At least one other product not yet discussed that may be used in food preservation systems could be a source of AIT exposure. A GRAS Notice was filed on behalf of CAREX, Inc. of Osaka, Japan on June 27, 2003, for a system also incorporating volatile oil of mustard (VOM) in a food preservation system designed for use with only

selected sub-segments of the diet. That GRAS Notice to FDA, numbered 133, resulted in an FDA "have no questions" letter on January 5, 2004.

Notification 133, filed by SRS International Corporation on behalf of CAREX, Inc., estimated that the use of VOM in the notified food preservation system would result in a total dietary load of up to 0.49 ppb of VOM (VOM) based upon the levels needed for food preservation and assumptions regarding potential market share. The notified system assumed a concentration of 95% AIT in the VOM. This dietary concentration would be equal to 1.47 mg/person for a person consuming a 3 kg diet.

The estimation of dietary exposure from the CAREX food preservation system was based upon the assumption that the maximum concentration of VOM in preserved food would be 9 ppm and that these exposures would be self-limiting. (This same notification estimated that flavoring uses resulted a maximum dietary exposure of 35 ppm, or 5.7 mg/person, which is less than that suggested in the National Research Council survey of 1970.)

Summary of Exposure Assessment

Dietary exposure to AIT may arise from various sources. Background exposure from consumption of raw or cooked Brassica species containing the glucosinolate, sinigrin, contribute the largest quantity of AIT. Other significant AIT contributions are from mustard condiments or flavorings. Estimates of AIT intake from various sources are shown in the table, below.

Table 2: Potential Sources of Exposure to AIT

Source of AIT	AIT Dietary Contribution (mg/person/day)	Percentage of Total
Brassica species (cauliflower, cabbage, Brussels Sprouts, broccoli)	11.84	58.9%
Mustard flavoring and condiments	6	29.8%
Horseradish flavoring and condiments	0.34	1.7%
MITSUBISHI Food Preservation System	1.464 (0.732)[1]	7.3%
CAREX Food Preservation System	1.47	7.2%
Total	20.11	100%

[1]Assuming 50% market share

Expected Increase in AIT Exposure from the New System

The Mitsubishi system incorporating AIT is similar to that notified by CAREX, Inc. in that it releases AIT into airspace found in storage bags and other food containers. Small amounts of AIT may deposit on the surface of the food or be absorbed into the food item. It is possible to generate a highly conservative overestimate of the maximum potential dietary exposure from this food preservation use by assuming that all of the AIT present in the Mitsubishi preservation system is released and is incorporated into the food that is contained in the storage bag. This worst-case exposure assessment assumes, unrealistically, that all of the highly volatile AIT gets into the food and also into the consumer, and that none of it is released (1) outside of the bag or other container during storage, or (2) upon the removal of the food item from the bag or container, or even (3) during any subsequent processing, cooking and consumption.

The following table shows the estimated dietary exposure to AIT from various forms of food packaging and food containers. It is assumed that 1.5 mg of AIT is used for bags, wraps, foil and containers, and that the Mitsubishi preservation system will achieve 100% market penetration for each of these items, another almost

certainly unrealistic overestimate. The number of products used per person per year is based upon recent survey data.

Table 3: AIT Exposure from MITSUBISHI Product Based Upon Use Survey

	AIT max (mg)	Average User/year	AIT Exposure Subtotal (mg/year)	AIT Exposure (mg/day)
Consumer				
Patch in Bags	1.5	16	24	0.07
Wrap	1.5	53.8	80.7	0.22
Foil	1.5	49.9	74.85	0.21
Containers	1.5	102.2	153.30	0.42
Foodservice				
Containers	1.5	103.60	155.40	0.43
Lids & Domes	1.5	30.66	46.00	0.13
		Total	**534.25**	**1.464**

Assuming a realistically conservative market share of 50% would reduce this exposure estimate to 0.732 mg/person/day.

A second, alternative approach to estimating dietary exposure is by using the default values that are routinely assumed by the FDA in the determination of dietary exposure to migration products from food packaging: assuming that 10 grams of food come into contact with one square inch of packaging material; that in the case of a typical bag the packaging material is 11.5 x 11.5 inches (264.5 in^2); and that 1500 micrograms of AIT may be present in the preservation system, the maximum food concentration would be:

$264.5\ in^2 \times 10\ g = 2645\ g$

$1500\ \mu g / 2645\ g = 0.567\ \mu g/g$

Assuming the consumption of a 3 kg diet, one patch per 264.5 in^2 results in a dietary intake of 1.7 mg/person/day if the entire diet is preserved using the Mitsubishi food preservation system. If the Mitsubishi food preservation system achieved 100% market share for polymer packaging and 40% of the diet may contact food packaging made from polymers, a total of 0.68 mg/person of AIT could potentially be added to the diet. This is approximately 46% of 1.464 mg, the value determined using the survey of consumer use of packaging and food storage systems.

Actual exposures would likely be significantly lower than those estimated using either approach because not all bags, wraps, foil or food containers in the case of the home use, or all polymers in the case of the foodservice or field uses would employ this food preservation. In other words, the likely market share of food packaging and food containers to use this system is not considered in either exposure assessment.

Safety of AIT:

Evaluation of the Toxicological Hazards of

Allyl Isothiocyanate

Introduction

Allyl isothiocyanate (AIT) (CAS 57-06-7) is the principal ingredient of VOM and AIT is often referred to as VOM. As discussed in the Exposure Assessment above, AIT is ingested in cruciferous vegetables, mustard and other common food items. This compound and the dietary sources have been evaluated for safety in several publications over the past 30 years. These evaluations have consistently concluded that AIT and VOM do not pose a risk to humans when consumed in the diet.

Mustard (Brassica species), including its extracts and oils, has several regulatory clearances. The GRAS status of the use of VOM in selected parts of the diet recently has been favorably reviewed by FDA in GRAS Notice 133 submitted by Carex, Inc. Further, both brown and yellow mustard (Brassica species) are GRAS when used as spices and other natural seasonings and flavorings. 21 CFR

182.10. The oil and extract of mustard (Brassica species) are also GRAS for its intended use in a regulation listing GRAS essential oils, oleoresins and natural extractives. 21 CFR 182.20. Mustardseed oil is an allowed component of the formulation of defoaming agents for the manufacturing of paper and paperboard, 21 CFR 176.210; and it is an approved substance for use in the production of or added to textiles and textile fibers, 21 CFR 177.2800.

In the early 1970s the available scientific literature for AIT was reviewed and evaluated by the Food and Drug Research Labs, Inc. (FDRL 1972) and the Federation of American Societies for Experimental Biology (FASEB 1975). Based on an extensive review of the literature the FASEB report conducted for FDA concluded that there was no evidence of any hazard to the public at the levels of use or consumption at that time (1975) or reasonably expected to occur in the future.

Background exposure to AIT from the diet, discussed in the Exposure Assessment, is more than an order of magnitude greater than the maximum exposure that may arise from uses proposed by Mitsubishi Corporation in this Notification.

Toxicology studies of AIT are available in the scientific literature. In 1999 the International Agency for Research on Cancer (IARC Monograph 73 [IARC 1999]) reviewed the existing literature for AIT, which included long-term bioassays with mice and rats (see discussion below) conducted by the National Toxicology Program (NTP [NTP 1982]). In addition, studies were reviewed relating to the metabolism and pharmacokinetics of AIT in humans and experimental systems and genotoxicity. The conclusion from the IARC evaluation was that "[T]there is inadequate evidence in humans for carcinogenicity of allyl isothiocyanate [AIT]" and "[T]there is limited evidence in experimental animals for the carcinogenicity of allyl isothiocyanate[AIT]" (IARC 1999). "Allyl isothiocyanate was not classifiable as to its carcinogenicity to humans (Group 3 [IARC 1999])."

Chronic toxicity

Carcinogenicity bioassays were conducted by the NTP with AIT in Fischer 344 rats and B6C3F1 mice. Both sexes of both species were dosed via gavage for 103 weeks. Results showed hyperplasia in the urinary bladder epithelium and an increased incidence of

transitional cell papillomas in male rats (0%, 4%, and 8% for the control, low dose (25 mg/kg/d) and high dose (50 mg/kg/d), respectively). The historical incidence of this tumor in male rats from the NTP and National Cancer Institute (NCI) Laboratories is 0.1% and the occurrence of this tumor in treated animals was clearly much greater than the historical control incidence. Female rats in this study showed only a single incidence of hyperplasia and papillomas at the high dose and an equivocal increase in subcutaneous fibrosarcomas[5] were observed in females only. The mice showed no increase in any tumor type at the same exposure levels (NTP 1982). The rat bioassay provides the only evidence of *in vivo* carcinogenicity of AIT. The relevance of this finding to humans, as discussed below, is questionable.

Metabolism and Excretion

Subsequent work designed to further evaluate the findings of the NTP study (NTP 1982) has shown that the tumors observed in male rats were due at least partially to species differences in metabolic pathways of AIT and in sex differences in urine flow and

[5] The incidence the fibrosarcomas is considered equivocal (HSDB 2003).

retention. The greater concentration of AIT in the urine may be associated with the chronic irritation in the urothelial tissue in the male rat bladder (Ioannou et al. 1984, Bollard et al. 1997). A two fold higher rate of urine flow and the resulting more frequent evacuation in female rats results in a higher concentration of AIT and AIT metabolites in the bladders of male rats (Bollard et al. 1997). The authors state that this longer retention time at higher concentrations may contribute to the observed higher incidences of transitional cell papillomas in male rats (Ioannou et al. 1984). Urinary volume is considered to influence epithelial carcinogenesis in the bladder through several mechanisms including dilution and concentration of endogenous and exogenous chemicals and metabolites (Cohen 1995).

Elimination of AIT was rapid (< 24 hours) and was predominantly (70-80%) in the urine of both rats and mice. Qualitatively both species shared similar metabolic profiles, although mice had more major metabolites and additional minor metabolites. The key difference was that the principal metabolite (74.9%-82%) in rats, the N-acetyl cysteine conjugate, N-acetyl-S-(N-allylthiocarbamoyl)-L-cysteine was a minor metabolite (8% -19.8%) in

mice. There was no observed association with irritation or hyperplasia in male or female mice.

Bollard and co-workers (1997) also evaluated the disposition of AIT in rats and mice. Species differences were observed in the rate and time of elimination, retention in the carcass and the moieties eliminated in urine. Dosed [^{14}C-]AIT (at 2.5 and 25 mg/kg/day) was 80% eliminated in mice by 96 hours. During this period the rate of elimination by rats was less than that found in mice and only 51.8% to 55.2% of [^{14}C-]AIT-derived radio label was eliminated in 96 hours. This resulted in a higher retention of the major metabolite(s) in the bladder for a longer period of time. In mice the primary metabolite in urine (up to 80%) was [^{14}C-]thiocyanate and in rats (approx. 75%) of the [^{14}C-]AIT-derived radioactivity was observed to be the N-acetylcysteine conjugate of AIT. Although species-related differences were observed in the metabolism of rats and mice, no marked sex-related differences were apparent in the pattern of metabolism for the two species.

Bollard and coworkers (1997) speculate that the combination of factors discussed above resulted in transition-cell papillomas in male rats that would not occur in humans. Humans excrete about 50% of

AIT in urine as the N-acetylcysteine conjugate (Jiao et al. 1994). Human intake of AIT is much lower than the 25 mg/kg/day dosed in the NTP study and subsequent work (Ioannou et al. 1984, Bollard et al. 1997) and the volume of human urine is much larger that in rats (approx. 1500 ml/d [Bollard et al. 1997]). In addition, the bladder urothelium of the rat may be more responsive to the irritation-induced neoplasia than that of other species. Based on these factors it is considered "...unlikely that such a secondary mechanism of carcinogenicity could operate in humans" (Bollard et al. 1997).

CAREX, Inc, in its GRAS Notice 133, made a similar analysis and reached a similar conclusion. See GRN 133 at 000109, as revised 10/14/2003.

Possible Mechanism of AIT-induced Bladder Cancer in Male Rats

A number of studies have been conducted to evaluate mechanism for the induction of hyperplasia and transitional cell papillomas in male rats observed in the NTP study of AIT (NTP 1982). The occurrence of benign bladder tumors in male rats, but not

female rats, would indicate a non-genotoxic mechanism of action, likely related to the chronic irritation of the urothelium of the bladder by AIT (Bechtel et al. 1998). AIT is a known irritant (HSDB 2003) and chronic irritation observed in the NTP study in male rats, but not female rats or mice (male or female), may be due to differences in urine volume and residence time in the bladder. As discussed above, urine volume is much lower in male rats and evacuation is less frequent, resulting a longer residence time for AIT-related compounds excreted in urine, e.g., N-acetylcysteine conjugate. AIT is believed to be released from conjugate metabolites in the urine during the residence time in the bladder and the AIT works directly on the urothelial cells. Thus the conjugates provide a mechanism for selective concentration of AIT in the bladder (Munday and Munday 2004) and the highest exposure level would be in male rats as discussed above.

Chronic irritation or inflammation of the bladder urothelium has been demonstrated to be linked to bladder cancer in rodents, and is characterized as predominantly transitional cell carcinomas (Burin et al. 1995) consistent with the results of the 1982 NTP study. In humans irritation and inflammation in the bladder may play a role in

carcinogenesis, but is associated more with squamous cell carcinoma than transitional cell carcinomas (Burin et al. 1995), indicating that rodents, specifically rats, may not be the most appropriate model for assessment of AIT-related bladder cancer in humans. The non-genotoxic, irritant-related transitional cell tumors in male rats do not appear to be directly relevant to human health risk assessment. In addition, AIT is reported to possibly serve as a mechanism for protecting humans from some types of cancer, including bladder cancer.

The Role of AIT in Preventing Bladder Cancer

In recent years AIT and cruciferous vegetables from which it is derived have been evaluated for its potential role as cancer prevention agent. AIT has been shown to be selectively cytotoxic and cytostatic to transformed human colorectal (HT29) tumor cells, *in vitro* (Musk and Johnson 1993); and has been reported to inhibit growth and survival of PC-3 human prostate cancer cell xenographs *in vivo* in mice (Srivastava et al. 2003). In the latter study the AIT-treated tumor mass showed a decreased rate of mitosis compared to the control tumors.

Increasing evidence shows that AIT-containing vegetables may provide a protective mechanism for bladder and other cancers. The results of a prospective epidemiological study indicated that dietary consumption of cruciferous vegetables provides a reduced risk of bladder cancer (Dominique et al. 1999). Intake of cruciferous vegetables (cabbage, cauliflower, Brussels sprouts, sauerkraut, Cole slaw, and kale) showed a decrease in relative risk (0.49 + 95% CI = 0.32 – 0.75) when the highest exposure group (> 3.4 servings per week) was compared to the lowest exposure group (< 1.8 servings per week (Dominique et al. 1999).

Diet-derived AIT has been shown to selectively induce microsomal enzymes in the urinary bladder and this enzyme induction may play a role in the decreased incidence of bladder cancer reported in epidemiology studies (Munday and Munday 2002, 2004). AIT has been shown to induce phase II metabolism in a range of tissues (liver, kidney, lungs, etc.), but induction was observed to be much stronger in the urinary bladder. Induction of microsomal enzymes in this tissue occurred at lower exposure levels than was observed in other tissues. The induction of phase II enzymes may result in a more rapid metabolism and excretion of carcinogenic

substances serving a protective function (Munday and Munday 2002, and 2004).

Other Studies

No data is available on the reproductive and developmental effects of AIT in humans. In experimental animals there is no evidence of either maternal toxicity or dose-related malformations in any species tested including rats and hamsters and avian embryos (IARC 1985, 1999 and FASEB 1975). The number of resorptions was observed to increase in a dose-related fashion (IARC 1985, 1999).

Genetic Toxicity

Genetic toxicity studies have been conducted in a range of species and test designs. Results are mixed with respect to bacterial mutation studies and *in vitro* studies with mammalian cell lines. The weight of evidence for *in vivo* studies does not support a conclusion that AIT is an *in vivo* genotoxicant.

In vitro

IARC (1999) reviewed a large number of genetic toxicity studies. Bacterial mutations studies were conducted with five strains of *Salmonella typhurium* (TA98, TA100, TA1535, TA1537 and TA1538) and *Escherchia coli* (WP67) with and without activation. Results were generally negative. *In vitro* studies with *allium cepa* root tip cells and with *Drosophila melanogaster* used extremely high exposure levels or were undocumented. In 12 studies with mouse lymphoma (L5178Y) cells, Chinese hamster ovary cells, and other *in vitro* mammalian test systems, results were mixed (IARC 1999). AIT did not cause dominant lethal mutations in mice or unscheduled DNA synthesis in the liver of rats exposed to AIT *in vivo.*

Subsequent work has suggested potential AIT genotoxicity in bacterial and mammalian cells *in vitro* (Kassie and Knasmüller 2000, Uhl et al. 2003). Radical scavengers have been shown to reduce the genotoxic effects of AIT *in vitro* (Kassie and Knasmüller 2000). Murata and coworkers (2000) have proposed a mechanism by which AIT and other isothiocyanates (ITCs) may cause oxidative damage to DNA in the presence of Cu II ion *in vitro*. "Such oxidative reactions

occurring either as a result of an endogenous oxidative phenomenon or from the administration of exogenous chemical carcinogens presumably are rapidly repaired by [DNA repair] mechanisms ...." (Klaassen 2001). Oxidative damage to DNA from AIT exposure could explain the genotoxicity observed in some *in vitro* studies where radical scavengers and DNA repair mechanisms were not present. The presence of radical scavengers and mechanism for repair of oxidative damage to DNA may explain the lack of observed AIT-induced genotoxicity in *in vivo* systems.

In vivo

In 1999, IARC reviewed and summarized seven *in vivo* studies and found that six of seven were negative. The single positive study was a *D. melanogaster* sex-linked recessive lethal mutation study[6], but AIT did not cause anueploidy in this test system. As summarized in IARC Monograph #73 (IARC 1999), no genotoxic effects were observed in a host-mediated assay, *Saccharomyces cerevisae* in the mouse peritoneal cavity. No chromosomal aberrations observed in rat bone-marrow cells and no dominant lethal mutations were

[6] This original study was not reviewed in preparation of the current document.

observed in rats *in vivo*. The conclusion reached in IARC Monograph #73 (IARC 1999) was that "... the available data do not allow a conclusion about the genotoxicity of [AIT] in experimental systems *in vivo*."

Subsequent work has confirmed that in *in vivo* mammalian systems AIT has not been found to be genotoxic and the weight-of-the-evidence is insufficient to regard this compound as a genotoxin capable of being carcinogenic *in vivo* (Bechtel et al. 1998). Livers from rats exposed to AIT did not show any unscheduled DNA synthesis activity (Bechtel et al. 1998). *In vivo* studies with AIT and other ITCs have been shown to induce phase II enzymes (e.g., quinone reductase and glutathione-S-transferase) in a range of tissues (Munday and Munday 2003, 2004). Induction was stronger in the urinary bladder and occurred in this tissue at a much lower AIT concentration (Munday and Munday 2002).

Conclusions of Toxicology Assessment

AIT has not been found to be an *in vivo* genotoxin based on a weight of evidence. Transition-cell papillomas in rat urinary bladder is not considered relevant to humans based on a non-genotoxic, irritant-

related mechanism involving differences in metabolism and the chemistry and physiology of the urine of these species. These conclusions are supported by epidemiological evaluations which show that consumption of foods containing AIT (cruciferous vegetables) is correlated with a significantly lower risk of bladder cancer in humans.

As demonstrated above, for decades, the US population has been exposed to AIT, a substance already recognized as GRAS in multiple contexts, through a variety of foods and food ingredients, at levels of approximately 19.65 mg/person/day or more, without any evident adverse health effects. The lack of health effects is supported by the extensive toxicological literature on AIT summarized above.

Mitsubishi's proposed use would add a small increment to the existing exposure, specifically, an estimated 0.732 mg/p/d, assuming 50% market share penetration by Mitsubishi's uses. The use of mustard as a direct food flavoring, and its recognition as GRAS in such contexts and for food preservation under GRAS Notice 133, further support the conclusion that the proposed use described in this

document is both safe and generally recognized as such by experienced scientists.

e. Statement of availability of information

Notifier has relied on published studies and generally accepted scientific data and information as the basis of its conclusions, and those of its Panel of Experts, about the safety and the general recognition of AIT in a food preservation system.

II. Identity of notified substance

1. Chemical name: Allyl isothiocyanate (AIT)

2. Chemical Abstracts Service: 57-06-7

3. Composition Specifications for food-grade material: The AIT employed in this system is to be of suitable purity for food contact, specifically

> 97% purity ("food grade")

Molecular weight	99.16
Specific gravity (15° C)	1.014-1.022 (Natural)

Vapor specific gravity	3.44
Vapor pressure (25° C)	5.2 mmHg
Vaporization rate	0.017-0.019 g/cm^2 – hr
Flash point	46.0° C

III. Self-limiting levels of use

The proposed use is self-limiting in that if AIT levels exceed a specified level, perceptible organoleptic changes in foods will occur. Mitsubishi has determined that the level at which the AIT will have the desired antimicrobial effect, 30-50 ppm, is below the level at which organoleptic changes occur.

IV. Basis of GRAS determination.

Mitsubishi believes its use of AIT is GRAS based on scientific procedures, 21 CFR Sec. 170.30(b), and convened a Panel of Experts qualified by scientific training and experience to evaluate the

safety of food, food additives and food ingredients. The experts have reviewed and evaluated the publicly available information summarized in this GRAS Notice. The above discussion and citations to generally available accepted scientific data, information, methods and principles relied upon, together with the anticipated consumption level for AIT, provide an ample basis to conclude that the use of AIT in a food preservation system is both safe and generally recognized as such by qualified experts.

The panel consisted of the following experts, whose curricula vitae are attached:

1. James F. Hobson, Ph.D., D.A.B.T.

Dr. Hobson is the principal environmental toxicologist of MorningStar Consulting, Inc. He has been a Diplomate of the American Board of Toxicology since 1989. He received his Master of Science in Toxicology degree from University of Arizona and his Ph.D. in biology from the University of Kentucky. Dr. Hobson has over 20 years of experience as an Environmental Toxicologist evaluating the exposure, effects and risks posed by chemicals in the environment.

2. Gary Burin, Ph.D., M.P.H., D.A.B.T.

Dr. Burin is the Director, Toxicology, Ecotoxicology and Risk Assessment Division of Technology Sciences Group, Inc. He has been a Diplomate of the American Board of Toxicology since 1985. He received his M.P.H. (concentration in toxicology) from the University of Michigan and his Ph.D. in biology from George Washington University. He is an internationally recognized expert in toxicology with twenty-five years of experience in the design, interpretation and use of toxicity studies.

Conclusion

Based on all the above information, Mitsubishi Corporation has concluded that its use of AIT as a shelf-life extension agent or anti-spoilage agent in a food container system for use in the home, in foodservice establishments, and in the farm field, is Generally Recognized as Safe within the meaning of 21 U.S.C. Sec. 321(s), and therefore exempt from the premarket approval requirements of Federal Food, Drug and Cosmetic Act, 21 U.S.C. Sec 301 et seq.

Sincerely,

(b)(6)

Eric F. Greenberg

Attachments

1. Authorization letter from Mitsubishi Foods Corporation for representation by Eric F. Greenberg

2. FASEB [Federation of American Societies for Experimental Biology]. (1975). "Evaluation of the Health Aspects of Mustard and Oil of Mustard as Food Ingredients" National Technical Information Service, No. PB-254528

3. National Toxicology Program. (1982). *Carcinogenesis Bioassay of Allyl Isothiocyanate (CAS No. 57-06-7) in F344/N Rats and B6C3F1 Mice (Gavage Study)*, NTP Technical Report No. 234 / National Technical Information Service, No. PB-83-144238.

4. Curriculum vitae of James F. Hobson, Ph.D., D.A.B.T.

5. Curriculum vitae of Gary Burin, Ph.D., M.P.H., D.A.B.T.

References

Bechtel, D., L. Henderson, and R. Proudlock. (1998). Lack of UDS activity in the livers of rats exposed to allylisothiocyanate. *Teratogenesis, Carcinogenesis, and Mutagenesis*, 18:209-217.

Bollard, M., S. Stribbling, S. Mitchell and J. Caldwell. (1997). The disposition of the allyl isothiocyanate in the rat and mouse. *Food and Chemical Toxicology*, 35:933-943.

Burin, G.J., H.J. Gibb, and R.N. Hill, (1995). Human bladder cancer: evidence for potential irritation-induced mechanism. *Food Chemical Toxicology*, 33(9):785-795.

Cohen, S. (1995). Role of urinary physiology and chemistry in bladder carcinogenesis. *Food and Chemical Toxicology*, 33(9):715-730.

Dominique, S.M., D. Spiegleman, S,K, Clinton, E. B. Rimm, W.C. Willett, E.L. Giovannucci. (1999). Fruit and vegetable intake and incidence of bladder cancer in a male prospective cohort. *Journal of the National Cancer Institute,* 91(7): 605-613.

Engel, E., C. Baty, D. LeCorre, I. Souchon and N. Martin (2002). Flavor-active compounds implicated in cooked cauliflower acceptance. *Journal of Agricultural and Food Chemistry* 50:6459-6467.

FASEB [Federation of American Societies for Experimental Biology]. (1975). "Evaluation of the Health Aspects of Mustard and Oil of Mustard as Food Ingredients" National Technical Information Service, No. PB-254528

FDRL [Food and Drug Research Labs., Inc.] (1972). GRAS (Generally recognized as safe) Food Ingredients: Oil of Mustard and Allyl Isothiocyanate. Food and Drug Research Labs., Inc. Maspeth, N.Y. Report # FDABF-GRAS-015, National Technical Information Service No. PB-221215.

HSDB (2003). Allyl isothiocyanate In: *Hazardous Substances Data Base* a database of the National Library of Medicine's TOXNET system (HTTP://toxnet.nlm.nih.gov.) Updated March 5, 2003.

Iannou, Y., L. Burka, and H. Matthews. (1984). Allyl isothiocyanate: comparative disposition in rats. *Toxicology and Applied Pharmacology* 75:173-181.

IARC (1999) "Allyl isothiocyanate" *IARC Monographs On The Evaluation Of Carcinogenic Risks To Humans,* 73: 37-48.

IARC (1985) "Allyl isothiocyanate" *IARC Monographs On The Evaluation Of Carcinogenic Risks To Humans*, 36: pp. 60 ff.

Jiao, D.,; C, Ho, P. Foiles, F. Chung. (1994). Identification and quantification of the N-acetylcysteine conjugate of allyl isothiocyanate in human urine after ingestion of mustard. *Cancer Epidemiology, Biomarkers And Prevention*, 3(6): 487-92.

Jiao, D., C. Conway, M. Wang, C. Yang, W. Koehl, and F. Chung. (1996). Inhibition of *N*-nitrosodimethylamine demethylase in rat and human liver microsomes by isothiocyanates and their glutathione, *L*-cysteine, and *N*-acetyl-L-cysteine conjugates. *Chem. Res. Toxicol.*, 9:932-938.

Kassie, F. and S. Knasmüller. (2000). Genotoxic effects of allyl isothiocyanate (AITC) and phenethyl isothiocyanate (PEITC). *Chemico-Biological Interations*, 127:163-180.

Heaney R.K. and G.R.Fenwick (1980). Glucosinolates in Brassica Vegetables. Analysis of Brussels Sprout (Brassica oleracea). *Journal of the Sciences of Food and Agriculture.* 31: 785-793.

Hrncirik, K. and J. Velisek (1997). Glucosinolate content of common Brassicacaseae family vegetables. *Potrav Vedy* 15: 161-172.

Michaud, D., D. Spiegelman, S. Clinton, E. Rimm, W. Willett, and E. Giovannucci. (1999). Fruit and vegetable intake and incidence of bladder cancer in a male prospective cohort. *Journal of the National Cancer Institute*, 91(7):1999.

Munday, R., and C. Munday. (2002). Selective induction of phase II enzymes in the urinary bladder of rats by allyl isothiocyanate, a compound derived from *Brassica* vegetables. *Nutrition and Cancer*, 44(1):52-59.

Munday, R., and C. Munday. (2004). Induction of phase II detoxification enzymes in rats by plant-derived isothyocyanates with sulforaphane and related compounds. *Journal of Agricultural Food Chemistry*, 52:1867-1871.

Murata, M., N. Yamashita, S. Inoue, and S. Kawanishi. (2000). Mechanism of oxidative DNA damage induced by carcinogenic allyl isothiocyanate. *Free Radical Biology & Medicine*, 28(5):797-805.

Musk, S., and L. Johnson. (1993). Allyl isocyanate is selectively toxic to transformed cells of the human colorectal tumor line HT29. *Carcinogenesis*, 14(10): 2079-2083.

National Toxicology Program. (1982). *Carcinogenesis Bioassay of Allyl Isothiocyanate (CAS No. 57-06-7) in F344/N Rats and B6C3F1 Mice (Gavage Study)*, NTP Technical Report No. 234 / National Technical Information Service, No. PB-83-144238.

Rouzaud, G., S.A. Young and A.J. Duncan (2004). Hydrolysis of glucosinolates to isothiocyanates after ingestion of raw or microwaved cabbage by human volunteers. *Cancer Epidemiology, Biomarkers and Prevention* 13:125-131.

Smith, T., S. Musk, I. Johnson. (1996). Allyl isothiocyanate selectively kills undifferentiated HT29 cells in vitro and suppresses aberrant crypt foci in the colonic mucosa of rats." *Biochemical Society Transactions*, 24(3): 381S.

Srivastava, S.J., D.X. Xiao, K.L. Lew, P. Hershberger, D.M. Kokkinakis, C.S. Johnson, D.L. Trump, and S.V. Singh. (2003). Allyl isothiocyanate, a constituent of cruciferous vegetables, inhibits growth of PC-3 human prostate cancer xenografts *in vivo*. *Carcinogenesis*, 24(10):1665-1670.

Tsao, R., Q. Yu, J. Potter, and M. Chira (2002). Direct and simultaneous analysis of sinigrin and allyl isothiocyanate in mustard samples by high-performance liquid chromatography. *Journal of Agricultural and Food Chemistry* 50: 4749-4753.

Uhl, M., B. Laky, E. Lhoste, F. Kassie, M. Kundi, and S. Knasmüller. (2003). Effects of mustard sprouts and allylisothiocyanate on benzo(a)pyrene-induced DNA damage in human-derived cells: a model study with the single cell gel electrophoresis/Hep G2 Assay.

Teratogenesis, Carcinogenesis, and Mutagenesis Supplement 1:273-282.

Yu E.Y., I.J. Pickering, G.N. George, R.C. Prince (2001). In situ observation of the generation of isothiocyanates from sinigrin in horseradish and wasabi. *Biochemica et Biophysica Acta 1527: 156-160.*

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July 26, 2005

Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food And Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Re: Authorization of counsel regarding NOTIFICATION OF CLAIM FOR GENERAL RECOGNITION OF SAFETY OF Allyl Isothiocyanate in a Food Shelf Life Extension and Anti-spoilage System

To the FDA:

Please take note that Mitsubishi-Kagaku Foods Corporation, with headquarters at 3-9, Ginza 1-chome, Chuo-ku, Tokyo, Japan, authorizes its attorney, Eric F. Greenberg, of the law firm Eric F. Greenberg, PC, 3500 Three First National Plaza, Chicago, Illinois 60602, to represent it and communicate on its behalf in all matters regarding the filing of its NOTIFICATION OF CLAIM FOR GENERAL RECOGNITION OF SAFETY OF Allyl Isothiocyanate in a Food Shelf Life Extension and Anti-spoilage System.

Sincerely,

(b)(6)

Mr. Akira Sato
Managing Director
Corporate Division
Mitsubishi-Kagaku Foods Corporation

PB254528

NTIS

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EVALUATION OF THE HEALTH ASPECTS OF MUSTARD AND OIL OF MUSTARD AS FOOD INGREDIENTS

FEDERATION OF AMERICAN SOCIETIES FOR EXPERIMENTAL BIOLOGY, BETHESDA, MD. LIFE SCIENCES RESEARCH OFFICE

1975

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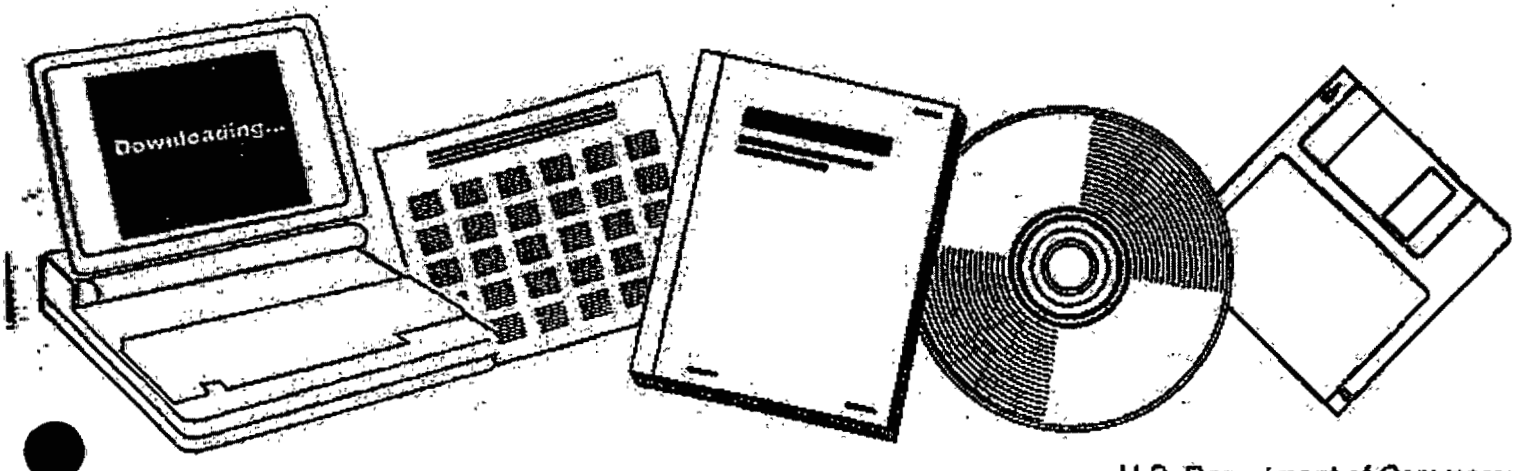

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SCOGS-16

EVALUATION OF THE HEALTH ASPECTS OF MUSTARD AND OIL OF MUSTARD AS FOOD INGREDIENTS

1975

Prepared for

Bureau of Foods
Food and Drug Administration
Department of Health, Education, and Welfare
Washington, D.C.

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Contract No. FDA 72-85

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Bethesda, Maryland 20014

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15. Supplementary Notes

16. Abstracts

This report, by a group of qualified scientists designated the Select Committee on GRAS Substances (SCOGS), provides an independent evaluation of the safety of mustard and oil of mustard, when used in food at present or projected levels of use.

17. Key Words and Document Analysis. 17a. Descriptors

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17b. Identifiers/Open-Ended Terms

APPROVED FOR UNLIMITED RELEASE

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DATE 5/24/76

WILLIAM L. JACKSON
DIRECTOR, FDA/PROC (NTIS)

17c. COSATI Field/Group

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NOTICE

This report is one of a series of evaluations of the health aspects of the Generally Recognized as Safe (GRAS) or prior sanctioned food substances being made by the Federation of American Societies for Experimental Biology (FASEB) under contract no. 72-85 with the Food and Drug Administration (FDA), U.S. Department of Health, Education, and Welfare. The Federation recognizes that the safety of GRAS substances is of national significance, and that its resources are particularly suited to marshalling the opinions of knowledgeable scientists to assist in these evaluations. The Life Sciences Research Office (LSRO), established by FASEB in 1962 to make scientific assessments in the biomedical sciences, is conducting these studies.

Qualified scientists were selected as consultants to review and evaluate the available information on each of the GRAS substances. These scientists, designated the Select Committee on GRAS Substances, were chosen for their experience and judgment with due consideration for balance and breadth in the appropriate professional disciplines. The Select Committee's evaluations are being made independently of FDA or any other group, governmental or nongovernmental. The Select Committee accepts responsibility for the content of each report. Members of the Select Committee who have contributed to this report are named in Section VII.

Tentative reports are made available to the public for review in the Office of the Hearing Clerk, Food and Drug Administration, after announcement in the Federal Register, and opportunity is provided for any interested person to appear before the Select Committee at a public hearing to make oral presentation of data, information, and views on the substances covered by the report. The data, information, and views presented at the hearing are considered by the Select Committee in reaching its final conclusions. Reports are approved by the Select Committee and the Director of LSRO, and subsequently reviewed and approved by the LSRO Advisory Committee (which consists of representatives of each constituent society of FASEB) under authority delegated by the Executive Committee of the Federation Board. Upon completion of these review procedures the reports are approved and transmitted to FDA by the Executive Director of FASEB.

While this is a report of the Federation of American Societies for Experimental Biology, it does not necessarily reflect the opinion of all of the individual members of its constituent societies.

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C. Jelleff Carr, Ph.D., Director
Life Sciences Research Office
FASEB

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SCOGS-16

EVALUATION OF THE HEALTH ASPECTS OF MUSTARD AND OIL OF MUSTARD AS FOOD INGREDIENTS

1975

Prepared for

Bureau of Foods
Food and Drug Administration
Department of Health, Education, and Welfare
Washington, D.C.

Contract No. FDA 72-85

Life Sciences Research Office
Federation of American Societies
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I. INTRODUCTION

This report evaluates the health aspects of using mustard and oil of mustard as food ingredients. The evaluation has been based partly on the information contained in a scientific literature review (monograph) furnished by FDA (1), which summarizes the world's scientific literature from 1920 through 1970.* To assure completeness and currency as of the date of this report this information has been supplemented by searches of over 30 scientific and statistical reference sources and compendia that are generally recognized as available; use of new, relevant books and reviews and the literature citations contained in them; consideration of current literature citations obtained through computer retrieval systems of the National Library of Medicine; searches for relevant data in the files of FDA; and by the combined knowledge and experience of members of the Select Committee and the LSRO staff. In addition, announcement was made in the Federal Register of December 19, 1974 (39 FR 43865 and 43866) that opportunity would be provided for any interested person to appear before the Select Committee at a public hearing to make oral presentation of data, information, and views on the health aspects of using mustard and oil of mustard as food ingredients. The Select Committee received one request for such a hearing but this request was withdrawn.

As indicated in the Food, Drug and Cosmetic Act [21 USC 321(3)], GRAS substances are exempt from the premarketing clearance that is required for food additives. It is stated in 21 CFR 121.1, revised April 1, 1974 that GRAS means general recognition of safety by experts qualified by scientific training and experience to evaluate the safety of substances on the basis of scientific data derived from published literature. This section of the Code also indicates that expert judgment is to be based on the evaluation of results of credible toxicological testing or, for those substances used in food prior to January 1, 1958, on a reasoned judgment founded in experience with common food use, and is to take into account reasonably anticipated patterns of consumption, cumulative effects in the diet, and safety factors appropriate for the utilization of animal experimentation data. FDA recognizes further (21 CFR 121.3) that it is impossible to provide assurance that any substance is absolutely safe for human consumption.

The Select Committee on GRAS Substances of LSRO is making its evaluations of these substances in full recognition of the foregoing provisions. In reaching its conclusions on safety the Select Committee, in accordance with FDA's guidelines, is relying primarily on the absence of

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substantive evidence of, or reasonable grounds to suspect, a significant risk to the public health, and realizes that a conclusion based on such reasoned judgment is expected even in instances where the available information is qualitatively or quantitatively limited. The Committee, aware that biological testing is dynamic, bases its conclusions on information now available; it cannot anticipate the results of experiments not yet conducted or those of tests that may be reconducted, using new technologies. These conclusions will need to be reviewed as new or better information becomes available.

In this context, the LSRO Select Committee on GRAS Substances has reviewed the available information on mustard and oil of mustard and submits its interpretation and assessment in this report, which is intended for the use of FDA in determining the future status of these substances under the Federal Food, Drug, and Cosmetic Act.

II. BACKGROUND INFORMATION

The mustard used in food is derived primarily from the seed of certain plants of the family Cruciferae. Brassica nigra (L.) Koch and B. juncea (L.) Czerniaew are the principal sources of black mustard seed from which the products called brown mustard and oriental mustard are prepared. B. nigra is cultivated chiefly in Italy and Holland; B. juncea is cultivated in northern India and southern Russia. B. alba (L.) Boiss or Sinapis alba (L.) is the principal source of white mustard seed from which the product called yellow mustard is prepared. White mustard is a native annual of southern Europe but is now widely cultivated in many temperate countries, chiefly Canada (1-5).

Mustard is used by the food industry in several forms: whole seed; ground seed meal; mustard cake (ground mustard seed from which a portion of the fixed oil has been expressed); mustard flour (ground mustard cake with hulls removed); and prepared mustard. Mustard flour and prepared mustard appear to be the forms most frequently used. Both may contain mustard from more than one source and, particularly in prepared mustard, may also contain a number of additional materials. In mustard flour, for example, blends of two or more mustard flours from different sources, together with mill fractions which the mustard miller has available, are often used to impart subtle flavors. In prepared mustards, as another example, food specifications permit their preparation from ground seed of white and/or black mustard, with or without mustard flour or mustard cake, together with vinegar and with or without addition of sweetening agents, salt, and such spices as turmeric, ginger, and black pepper. Prepared mustards contain about 10 to 15 percent solids (5, 6).

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The characteristic flavor of brown mustard (described as "nose heat" or "horseradish-like bite") is due to allyl isothiocyanate. This is present in the seed as part of the glucoside sinigrin, from which it is released, together with glucose and potassium bisulfate, by the action of the enzyme myrosinase which also is present in the seed. The allyl isothiocyanate thus released is sufficiently stable chemically to be readily steam distillable. The distilled product, known variously as oil of mustard, volatile oil of mustard, and natural allyl isothiocyanate, is the most commonly used commercial mustard oil. Yield of the oil from the seed ranges from 0.5 to more than 1.0 percent. As prepared, it consists of more than 90 percent allyl isothiocyanate; the remainder is chiefly allyl cyanide and carbon disulfide. However, most of the allyl isothiocyanate used in food is prepared synthetically. Allyl isothiocyanate volatilizes easily, is lost from open containers in four to six months, and is susceptible to decomposition in air and light (4, 5, 7).

The corresponding constituent of yellow mustard is p-hydroxybenzyl isothiocyanate. It is present in the seed as part of the glucoside sinalbin from which it is released, together with glucose and sinapine bisulfate, by the action of myrosinase. Sinalbin mustard oil is only sparingly volatile with steam but can be separated by solvent extraction. About 3 or 4 percent of p-hydroxybenzyl isothiocyanate is present in the defatted flour of white mustard seed (8, 9). When freshly prepared, sinalbin mustard oil is strongly pungent with a mildly burning taste, described as "mouth heat." However, its principal constituent, p-hydroxybenzyl isothiocyanate, is highly unstable, and hydrolyzes in a matter of hours, at room temperature, to p-hydroxybenzyl alcohol, di (p-hydroxybenzyl) disulfide, and p-hydroxybenzyl cyanide (4, 5, 7, 10-13). According to Bice (5) and Baróthy (14), these hydrolytic products do not contribute significantly to the flavor of prepared yellow mustard; added spices and other constituents of the seed such as sinapine (the choline ester of sinapic acid) are the chief sources of its flavor.

Some of the reactions referred to in the preceding paragraphs are given below:

Sinigrin (Potassium myronate) $\xrightarrow{\text{myrosinase}}$ $CH_2{=}CH{-}CH_2{-}N{=}C{=}S$ + glucose + potassium bisulfate
allyl isothiocyanate

Under some conditions the following may also be formed:

$CH_2{=}CH{-}CH_2{-}S{-}C{\equiv}N$
allyl thiocyanate

$CH_2{=}CH{-}CH_2{-}C{\equiv}N$
allyl cyanide

$S{=}C{=}S$
carbon disulfide

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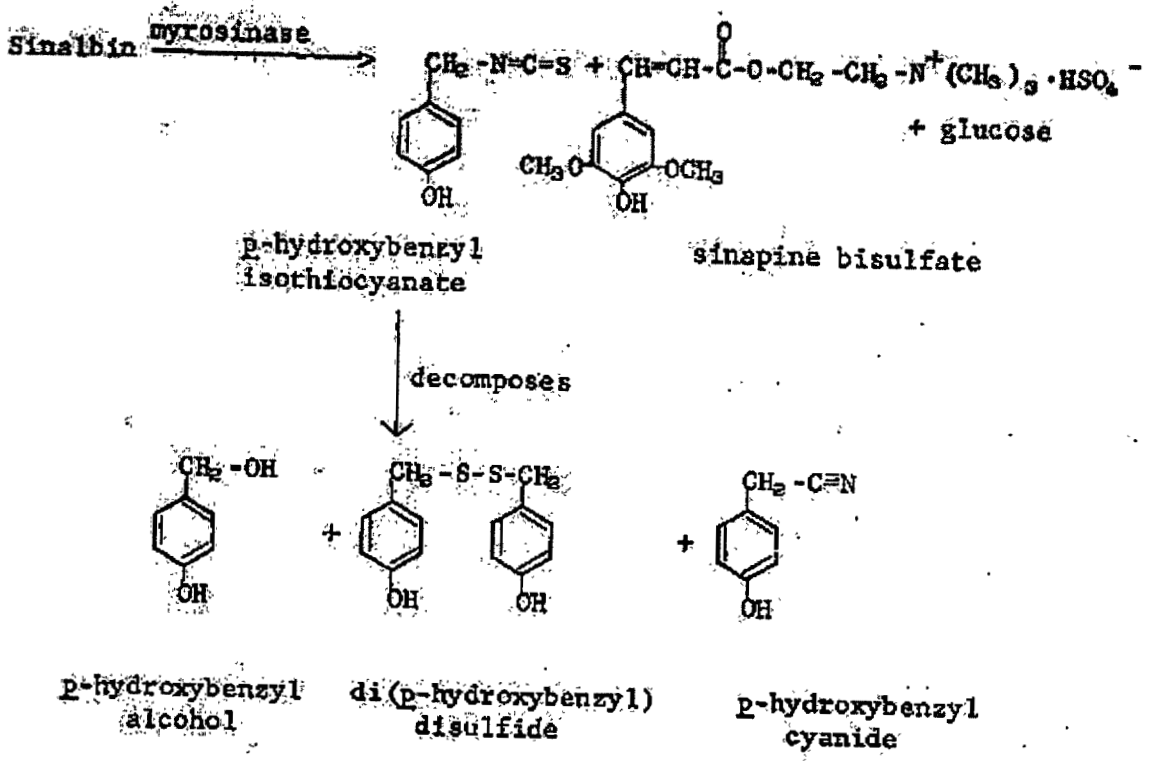

It is specified in the Food Chemicals Codex that food grade "mustard oil, volatile (allyl isothiocyanate)," whether from natural or synthetic sources, should assay not less than 93 percent allyl isothiocyanate, and conform to stated ranges with respect to distillation temperature, refractive index and specific gravity. Maximum limits are specified for arsenic and heavy metals (15). The Codex contains no specifications for p-hydroxybenzyl isothiocyanate or other mustard products.

A number of other plant seeds, mainly Cruciferae, contain isothiocyanate oils that are referred to as mustard oils (7). There is no record of their commercial use, but the similarity of many of them to those described above has been established. For example, in rape seed, B. napus (L.), the characteristic ingredient of its volatile oil is allylcarbinyl isothiocyanate (16).

A comprehensive survey by a National Research Council (NRC) subcommittee (17) reports that brown and yellow mustard and allyl isothiocyanate are used as flavoring in food products as indicated in Table I.

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Table I

Use of Mustard and Allyl Isothiocyanate in Foods

Food category	Brown mustard		Yellow mustard		Allyl isothiocyanate	
	Usual	Max.	Usual	Max.	Usual	Max.
	ppm		ppm		ppm	
Condiments, relishes, salt substitutes	82,071	101,503	60,995	124,274	17	26
Fats and oils	9,838	18,344	3,470	8,541	50	50
Nuts, nut products	6,200	10,000				
Gravies, sauces	4,859	5,170	235	610	46	201
Baked goods, baking mixes	3,000	3,000	590	1,200	14	29
Snack foods	820	820	13,506	15,863		
Processed vegetables juices	179	216	1,345	2,013		
Meat products	9	***	10,163	10,738	35	61
Fish products			1,000	2,000		
Soups, soup mixes			30	30		
Frozen dairy desserts, mixes					2	3
Soft candy					2	3
Gelatins, puddings, fillings					1	2
Beverages Type I (nonalcoholic)					1	2

Blanks in the table mean that the substance is not added to the foods indicated; asterisks (***) in the table mean that (a) the substance is used in a processing phase of the foods indicated but residual levels in the food product are negligible or unknown, or (b) the substance is used in the foods indicated but usage levels were not provided by industry, or (c) the substance is in the foods indicated but the levels were considered to be reported incorrectly (see explanatory notes on exhibit 40 of reference 17).

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Domestic production of mustard seed (color not specified) was 5,276,621 pounds (2,398,464 kg) in 1969; 60 percent was grown in California and Montana (18). Bureau of the Census statistics also show that imports of whole mustard seed (color not specified) increased about threefold between 1965 and 1972 to about 104 million pounds in 1972. Imports of ground and prepared mustard increased slightly less than twofold over the same period, reaching just under 2 million pounds in 1972 (19). According to other estimates (5) about 57 million pounds of mustard seed (more than 75 percent white seed) were used by the U.S. food industry in 1972. The Select Committee has no information from which to determine if there has been significant change over the past decade in the mustard content of the food categories shown in Table I.

III. CONSUMER EXPOSURE DATA

The survey of the NRC subcommittee has provided information on the possible daily human intake of mustard in the diet (Table II) for various age groups (17). The Select Committee has converted these figures to possible intake per kilogram of body weight.

The NRC subcommittee has pointed out that its calculations of GRAS substance intakes in most cases are overstated, often by considerable margins.* The Select Committee believes that this is probably so in the case of mustard products. The NRC subcommittee has also provided data (17) to show that approximately the following amounts were used annually by the food industry in the United States in 1970: yellow mustard, 48.5 million pounds (22 million kg); brown mustard, 3.2 million pounds (1.45 million kg); allyl isothiocyanate, 33,000 pounds (15,000 kg). These figures have been derived from those given by the NRC subcommittee which they estimate to represent about 60 percent of actual use. Using a population of 210 million, these poundages would provide for an average per capita daily consumption of only 287, 18, and 0.2 mg of yellow mustard, brown mustard, and allyl isothiocyanate, respectively, rather than the

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*An explanation for such overstatements is detailed in Section XI, "Significance and Use of Data in Safety Evaluations," of the NRC subcommittee's report (17). The Select Committee finds this explanation reasonable, and concurs in the first recommendation in Section XII of the same report, that "in order to conduct a more accurate survey on the intake of substances used in food processing, food consumption data collected specifically for this purpose are needed."

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Table II

Possible Daily Intake of Mustard and Allyl Isothiocyanate

Total intake, mg								Substance	Intake, mg per kg body weight[1]							
0-5 mos		6-11 mos.		12-23 mos.		2-65+ yrs.			0-5 mos.		6-11 mos.		12-23 mos.		2-65+ yrs.	
Av	Max	Av	Max	Av	Max	Av	Max		Av	Max	Av	Max	Av	Max	Av	Max
17	47	324	856	606	1234	1622	3126	Mustard, yellow[2]	3.4	9.4	40.5	107.0	55.1	112.2	27.0	52.1
16	30	204	523	498	1131	1396	2892	Mustard, brown[3]	3.2	6.0	25.5	65.4	45.3	102.7	23.3	48.2
<1	<1	1	3	2	5	6	12	Allyl isothiocyanate	<0.1	<0.1	0.1	0.4	0.2	0.4	0.1	0.2

[1] Calculations based on an average weight of 60 kg for an adult (20), and the following estimated weights of infants by age groups: 0-5 mos., 5 kg; 6-11 mos., 8 kg; and 12-23 mos., 11 kg (21).

[2] Assumed to be flour prepared from white mustard seed; estimated to contain 1 to 2 percent mustard oil consisting chiefly of p-hydroxybenzyl isothiocyanate and/or its decomposition products.

[3] Assumed to be flour prepared from black or brown mustard seed; estimated to contain 1 to 2 percent mustard oil consisting chiefly of allyl isothiocyanate.

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1622, 1396, and 6 mg given in Table II. The lower intake figures, based on poundage used, appear the more plausible since import and domestic production statistics show that some 90 million pounds of whole and ground mustard seed are available annually (18, 19). These statistics do not differentiate between white and brown mustard seed. However, in converting this 90 million pounds of whole and ground seed to the mustard flours used in food, loss of the fatty fraction would reduce the weight used to a figure closely approximating that indicated by the NRC subcommittee for total poundage of yellow and brown mustard reported to be used annually by the food industry (51.7 million pounds).

On the basis of these considerations, the Select Committee regards the figures in Table II as levels that are severalfold greater than those likely to be achieved by any of the age groups. It should also be noted in this connection that the isothiocyanates of brown and yellow mustard are either highly unstable or volatile, making the effective amount present in most foods as consumed unpredictably less than the amount added.

IV. BIOLOGICAL STUDIES

A. Brown mustard and its constituents

The oral LD_{50} of allyl isothiocyanate from brown mustard seed for the rat is reported to be 339 mg per kg (22); the subcutaneous LD_{50} for mice, 80 mg per kg (23). For rats, the oral LD_{50} of allyl cyanide, reported to be a constituent of the essential oil of brown mustard, is 115 mg per kg (24); for rabbits and oral lethal dose of carbon disulfide, also reported to be a constituent of the essential oil of brown mustard, appears to be between 0.47 and 3.5 g (about 0.23 to 1.75 g per kg) (25).

Rats fed a diet containing allyl isothiocyanate (0.02 mg per kg body weight) for 70 days showed an increased level of fatty acid esters and cholesterol in the blood serum. Histologically, some fatty degeneration of the liver was noted but this was most marked when the allyl isothiocyanate was fed in combination with ethyl alcohol (15 ml of a 20 percent ethanol solution per kg animal weight) (26). Allyl isothiocyanate is reported to depress rate of growth slightly when fed to young rats at a level of 0.1 percent in the diet (estimated dose level, about 400 mg per kg) for 5 weeks; a more pronounced decrease in the growth rate occurred when the same amount of allyl isothiocyanate was administered by gavage rather than fed for an additional four weeks (27). In adult rats fed 2 mg of

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allyl isothiocyanate per kg body weight it was observed that blood clotting time was reduced, liver succinic dehydrogenase activity was increased, and the activity of both xanthine oxidase and kidney D-amino acid oxidase was decreased significantly when measured 4 hours after feeding (27).

Two groups of weanling Osborne-Mendel rats (5 males and 5 females in each group) received a daily dose (by stomach tube) of 20 mg per kg and 50 mg per kg body weight of allyl isothiocyanate respectively, for 20 days. Organ and tissue examination showed the following histopathology: slight to moderate epithelial hyperplasia of the nonglandular part of the stomach with acute to subacute ulcers from 2 to 6.5 mm across in all animals at the higher dose and in half of the animals at the lower dose; and minor inflammatory foci in the liver at the higher dose level. However, the same authors reported that no effects were noted after feeding weanling rats for 26 weeks on a diet containing up to 10,000 ppm (about 500 mg per kg per day) of allyl isothiocyanate (28). The diet was prepared daily owing to the volatility of the compound. It is noted that this dosage is significantly higher than the LD_{50} reported from the same laboratory (22); reasons for the variation are not clear.

The Select Committee was unable to find reports of long term animal studies on brown mustard or its constituents, or of studies on the absorption or excretion of these substances.

Teratologic evaluation was made of a sample of allyl oil of mustard supplied by FDA and known to have been prepared from black mustard seeds (B. nigra L.). The oral administration of up to 18.5 mg per kg body weight daily to pregnant rats (6th through 15th days of gestation), up to 23.8 mg per kg to pregnant hamsters (6th through 10th days of gestation), and up to 12.3 mg per kg to pregnant rabbits (6th through 18th days of gestation), had no clearly discernible effect on nidation or on maternal or fetal survival (29). The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls. The same result was obtained with pregnant mice at dosage levels up to 6.0 mg per kg (administered on the 6th through 15th days of gestation) but at a level of 28.0 mg per kg there were indications (not statistically significant) of increased fetal deaths and resorptions. These investigators concluded that oil of mustard, at this high level may be fetotoxic in the pregnant mouse without being teratogenic.

Studies of the toxicity and teratogenicity of mustard oil (allyl isothiocyanate) in avian embryos revealed that it was not teratogenic under the conditions employed (30). Doses up to 16 mg per kg were injected into the air cell or the yolk of fertile eggs before incubation or after 96 hours

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of incubation. The occurrence of abnormal embryos and those showing head, skeletal, visceral and limb abnormalities was not increased with the administration of mustard oil under any of these conditions. In the same series of experiments, mustard oil was found to be toxic to avian embryos when injected into the air cell of unincubated fertile eggs or after 96 hours of incubation, at a level of 0.6 mg per kg, and embryo mortality was increased with doses as low as 0.04 mg per kg when injected into the yolk.

Mutagenicity studies of mustard oil (allyl isothiocyanate) in which several assay procedures were employed, were uniformly negative (31). The assay procedures and the doses used were as follows: host-mediated assay using two strains of Saccharomyces cerevisiae at oral doses up to 130 mg of mustard oil per kg in mice; cytogenetic assay, in vivo, involving observation of aberrations in bone marrow metaphase chromosomes of rats administered up to 100 mg of mustard oil per kg orally; cytogenetic assay, in vitro, involving observation of aberrations in anaphase chromosomes of human embryonic lung cells in tissue culture exposed to levels up to 10 ug of mustard oil per ml; dominant lethal assay in rats using dosages of mustard oil up to 100 mg per kg.

No reports have been found on the testing of allyl cyanide or carbon disulfide for teratogenicity.

Allyl isothiocyanate, applied to the skin of female Sutter albino mice as a 50 percent solution in acetone with 2 percent corn oil twice weekly for 24 weeks, produced papillomas in 10 percent of the animals and carcinomas in 1 animal (32). A 0.003 normal solution of allyl isothiocyanate in acetone painted on the skin of mice twice weekly for 5 months produced no tumors (33). No skin tumors were found after 11 months in mice painted three times weekly with allyl isothiocyanate (34). No reports have been found concerning the potential carcinogenicity of orally administered allyl isothiocyanate.

Carbon disulfide in aqueous solution (concentration not reported) administered orally to dogs and rabbits, produced no tumors in three months (35). No reports have been found in which the carcinogenic potential of allyl cyanide has been studied by any route.

Based on the eating habits of a number of his patients who had experienced coronary attacks, Blair in 1955 (36) expressed the opinion that allyl isothiocyanate is the underlying cause of atheromatous sclerosis in a majority of cases. He has suggested that restriction of the "hot" condiments - e.g., mustard, pepper, and ginger - offers a means for prevention of essential hypertension and coronary disease. The editor of the Ohio State Medical Journal in which this article appeared, noted that the author's conclusions, while not substantiated, "represents a different viewpoint which may, or may not, be borne out in the experience of others."

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B. Yellow mustard and its constituents

The subcutaneous LD_{50} of benzyl mustard oil (presumed to consist principally of p-hydroxybenzyl isothiocyanate from yellow mustard seed) for mice is stated to be 150 mg per kg (23). No data have been found on acute studies of orally administered benzyl mustard oil or its isothiocyanate. Acute toxicity studies, by any route of administration, of sinapine, p-hydroxybenzyl alcohol, di-(p-hydroxybenzyl) disulfide, p-hydroxybenzyl cyanide, or p-hydroxybenzyl amine, which are reported to occur in yellow mustard or to be present as decomposition products of p-hydroxybenzyl isothiocyanate have not been found in the literature.

The Select Committee was unable to find reports of short or long term studies on yellow mustard or its constituents, or studies on the absorption or excretion of these substances.

Only one experiment has been found that concerns the metabolic fate of mustard. In man, Perry et al. (37) fed 100 g of mustard to a male adult over a period of 1.5 hours and collected urine 24 hours after the start of consumption. Chromatographic analysis of the urine revealed the presence of p-hydroxybenzyl amine and an unidentified amine, metabolites that were not detectable in the urine when mustard was excluded from the diet. The mustard used was "prepared mustard, made from mustard seed, turmeric and other spices," but the source of the seed was not indicated. Since the allyl isothiocyanate of brown mustard could not conceivably give rise to a p-hydroxybenzyl metabolite, it can be reasoned that the mustard fed in this experiment was probably of the yellow variety and that the source of the p-hydroxybenzyl amine excreted could either be a separate constituent of white mustard seed as reported by Larsen (38) or could arise as a metabolite of p-hydroxybenzyl isothiocyanate; p-hydroxybenzyl amine has also been found in barley seeds (39).

No reports have been found on the carcinogenicity, teratogenicity, or mutagenicity of white mustard seed, or of its reported constituents, or of the degradation products of its constituents.

C. Mustard (variety unidentified)

The oral LD_{50} of the volatile oil of mustard (source not indicated) for the rat was reported to be 148 mg per kg; 50 mg per kg per day was tolerated by rats for eight weeks without noticeable injury (40).

For 30 days rats were fed a basal diet containing 10 percent mustard cake (source not indicated) supplemented with 20 percent of one of a number of vegetable oils including mustard oil (source not

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indicated) (41). It is evident that the mustard cake used in the basal diet was mustard seed from which the fatty acid glycerides had been expressed, leaving some or all of the essential oil; and that the mustard oil used was the fatty acid glycerides of mustard seed containing some or all of the essential oil. Serum lipid levels decreased in animals receiving the mustard oil during the early part of the feeding, but subsequently the level showed a steady upward trend. Mustard oil also produced consistent and progressive increases in the liver lipid level. Similar effects were noted with some of the other vegetable oils tested, making it appear that the effects noted with mustard oil were not due to its essential oil constituents. No evidence of toxicity to the animals subjected to this regime was reported by the investigators (41).

In one study, the nutritive contribution of mustard oil (expressed) appeared to be limited to its fat content. The source of the mustard oil was not indicated. Feeding tests with rats showed that this mustard oil (assumed to be the expressed fatty acid glycerides of mustard seed, containing some or all of the essential oil) was entirely devoid of vitamin A activity, and tended to inactivate vitamin A when mixed with other foods. The tests also showed that, as a fat, mustard oil was as nutritious as other fats, provided adequate vitamin A intake was assured from other sources (42).

The possibility of synergism between mustard oil (source not indicated, but presumed from the context of the report to be expressed mustard oil containing the fatty acid glycerides as well as the essential oil) and cholesterol was studied by including 10 percent of mustard oil in the feed of growing chicks, with or without one percent cholesterol for eight weeks. Chicks on the diet containing mustard oil showed an increase in the level of nonesterified fatty acids in the blood plasma but no change in other plasma lipids. Chicks on diets containing both cholesterol and mustard oil showed an increase in total plasma cholesterol, β-lipoprotein cholesterol, triglycerides, and nonesterified fatty acids. The extent of hypercholesterolemia did not seem dependent on the degree of saturation of the mustard oil (43). A subsequent report from the laboratory of one of these investigators extended these observations to effects on portions of the aorta by feeding diets containing 10 percent mustard oil or other oils, with or without one percent cholesterol, to growing chicks for eight weeks (44). When such oils were fed without cholesterol, there was an increase in phospholipids in the aorta but no change in cholesterol; whereas cholesterol, with or without the oils, increased both cholesterol and phospholipids in the aorta. Further, the hexosamine content of the aorta decreased regardless of whether cholesterol was fed, implying a concomitant decrease in acid mucopolysaccharides. The hydroxyproline content of the aorta increased when both oils and cholesterol were fed, indicating that arterial collagen had increased. The latter effect seemed

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to be proportional to the degree of hypercholesterolemia. Severity of changes were found to be unrelated to either the cholesterol level in the plasma or to the degree of unsaturation of the oils fed. In all respects, mustard oil behaved no differently than coconut, sesame, and hydrogenated peanut oils (44).

Observations were made of the short term physiological effects due to mustard (source not indicated), lemon, curry, paprika, and sugar fed as condiments in test diets of healthy young men. It was noted that increase in salivary flow was of about the same magnitude for all of these substances, while mustard and paprika were least effective in increasing salivary amylase activity (45).

Bandyopadhyay and Banerjee (46) fed up to 20 percent of various vegetable oils, including what is presumed to be expressed oil of mustard of unspecified source, with and without cholesterol, in the diet of rhesus monkeys for nine months. In the animals fed diets containing the mustard oil, diminished amounts of cholesterol deposits were found at autopsy in the thoracic and abdominal aortas, the brain, and the skin, while increased amounts of cholesterol were found in the liver. In a similar study, also with rhesus monkeys, Banerjee et al. (47) fed cholesterol and various vegetable oils (up to 20 percent of the diet) for six months. Periodic observations showed that the level of plasma cholesterol was raised and fecal cholesterol was reduced to the same degree, indicating that the vegetable oils probably facilitated intestinal absorption of cholesterol.

Twenty human adult volunteers were fed a breakfast containing 17 percent by weight (about one g per kg) of one of six different vegetable or animal fats, including mustard oil (source not identified but evidently the expressed oil containing the fatty acid glycerides as well as some or all of the essential oil). A blood sample, taken four hours after breakfast, was analyzed. The experiment was repeated on each of five successive days. Feeding mustard oil was found to have no effect on platelet content, serum cholesterol, Stypvan* time, or the fibrinolysing property of the blood; whole blood clotting time was reduced as it was after feeding any of the fats, and fibrinogen content decreased, as it also did after feeding sesame oil; platelet adhesiveness increased as it also did after feeding groundnut, sesame or coconut oils (48).

Two mustard oils were tested for carcinogenic activity in XVII x C57 (black) hybrid mice by oral, cutaneous, subcutaneous, and intraperitoneal routes. There was no evidence of malignancy due to

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*Stypvan: a preparation of Russell's viper venum, used as a thromboplastic agent in the one-stage prothrombin time test.

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either oil at the site of treatment or in the remote organs even though the animals were observed for their complete life span of over two years (49). One of the oils was obtained from the Indian Agricultural Research Institute, New Delhi, and the other from the Raj Oil Mills, Bombay. Direct inquiry by the Select Committee of the authors and the Indian Agricultural Research Institute provided little additional information concerning the identity of these oils; the IARI sample was of unknown origin and the Raj sample was a market sample and believed to be a mixture. In more recent work the same investigators (50, 51) found no evidence of carcinogenicity in mice administered laboratory expressed refined mustard oil (seed source not indicated) by stomach tube five days each week for their life span. Under the same conditions, one of 27 mice fed a market sample of mustard oil (seed source not indicated) developed transplantable gastric carcinoma in 13 months.

V. OPINION

The two mustards (brown and yellow) commonly used in the United States are derived from two Brassica species that differ with respect to the major chemical constituents of their essential oils. The available information shows that the characteristic isothiocyanates, other known constituents, and decomposition products of the essential oils of both mustards, have low orders of oral toxicity in experimental animals. Further, there is no reported evidence that orally administered doses of these constituents are carcinogenic, teratogenic, or mutagenic. However, interpretation of the available information suffers from a general lack of specificity on the part of investigators concerning the identity of the particular mustard used in their experiments, and from the fact that the major constituents of the essential oils are either very volatile or unstable, making it speculative how much of each of these substances was actually present in the preparation tested. The industrial practice of using mixtures of mustards to impart desired flavors compounds the problem of relating the results of toxicological studies to conditions that may exist in foods. For the same reasons, the amounts of the essential mustard oil constituents actually present in foods at the time of consumption are unpredictably variable, but must be less than the amounts calculated from the reported daily human intake levels of mustard.

The Select Committee has made an exhaustive search for information relevant to the health aspects of mustard and mustard oils as food ingredients and is confident that the literature evaluated in this report is representative and significant as a basis for judgment. However, because

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of the long history of use of the mustards in or on foods without apparent adverse effects on man, and because, as a consequence, they have been generally recognized as safe, there have been few well-defined toxicological studies. The Select Committee is of the opinion that the mustards are examples of substances where more definitive toxicological studies on representative samples of the products actually used by the food industry would be useful in confirming the apparent absence of adverse health effects when they are used in or on foods. Moreover, in products like the mustards, which consist of variable mixtures of mustard seeds containing several chemical entities, the toxicological study of the products as actually used, at levels that approximate or exceed those now in current use, would be of greater significance and usefulness than the separate study of constituents known or assumed to be present.

The Select Committee has weighed the foregoing data and concludes that:

> There is no evidence in the available information on allyl isothiocyanate, p-hydroxybenzyl isothiocyanate, and brown and yellow mustard that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when they are used at levels that are now current or that might reasonably be expected in the future.

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VI. REFERENCES CITED

1. Food and Drug Research Laboratories, Inc. 1972. Monograph on oil of mustard and allyl isothiocyanate. Submitted under DHEW contract no. FDA 72-103. Maspeth, N.Y. 65 pp.

2. Informatics, Inc. 1973. Monograph on p-hydroxybenzyl isothiocyanate. Submitted under DHEW contract no. 72-104. Rockville, Md. 9 pp.

3. Parry, J.W. 1969. Mustard. Pages 199-200 in J.W. Parry. Spices, vol. 1. Chemical Publishing Co., New York, N.Y.

4. Guenther, E. 1952. Oil of black mustard seed (oil of mustard). Pages 55-56 in E. Guenther, ed. The essential oils, vol. 6. Robert E. Krieger Publishing Co., Inc., Huntington, N.Y.

5. Bice, C.W. May 29, 1973. Letter to L.F. Mansor, Food and Drug Administration, from C.W. Bice of the R.T. French Company, 1 Mustard Street, Rochester, N.Y.

6. Hart, F.L., and H.J. Fisher. 1971. Mustard. Pages 342-344 in F.L. Hart and H.J. Fisher, Modern food analysis. Springer-Verlag, New York, N.Y.

7. Kjaer, A. 1956. Naturally derived isothiocyanates (mustard oils) and their parent glucosides. Fortschr. Chem. Org. Naturst. 18:123-176.

8. Josefsson, E. 1968. Method for quantitative determination of p-hydroxybenzyl isothiocyanate in digests of seed meal of *Sinapis alba* L. J. Sci. Food Agr. 19:192-194.

9. Raghavan, B., M.L. Shankaranarayana, S. Nagalakshmi, and C.P. Natarajan. 1971. Volumetric determination of p-hydroxybenzyl isothiocyanate in sinalbin (p-hydroxybenzylglucosinolate) and in white mustard seed (*Sinapis alba* L.). J. Sci. Food Agr. 22:523-525.

10. Kawakishi, S., and K. Muramatsu. 1966. Studies on the decomposition of sinalbin. Part I. The decomposition products of sinalbin. Agr. Biol. Chem. 30:688-692.

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11. Kawakishi, S., M. Namiki, H. Watanabe, and K. Muramatsu. 1967. Studies on the decomposition of sinalbin. Part II. The decomposition products of sinalbin and their degradation pathways. Agr. Biol. Chem. 31:823-830.

12. Baróthy, J., and H. Neukom. 1965. Synthesis of p-hydroxybenzyl-isothiocyanate and its isolation from white mustard seeds. Chem. Ind. 74:308-309.

13. Salkowski, H. 1889. Ueber einige derivate der p-oxyphenylessigsaüre und das ätherische oel des weissen senfs. Ber. Deut. Chem. Ges. 22:2137-2144.

14. Baróthy, J. 1964. Biologische wirkung von isothiocyanaten. Pages 48-49 in J. Baróthy, Uber p-hydroxybenzyl-isothiocyanat, das senfól aus samen von Sinapis alba L. Prom. Nr. 3537. Juris-Verlag, Zurich, Switzerland. (In German; translation supplied with reference no. 2).

15. National Research Council. 1972. Allyl isothiocyanate. Pages 31-32 in Food chemicals codex, 2nd ed. National Academy of Sciences, Washington, D.C.

16. Ettlinger, M.G., and J.E. Hodgkins. 1955. The mustard oil of rape seed, allylcarbinyl isothiocyanate, and synthetic isomers. J. Amer. Chem. Soc. 77:1831-1836.

17. Subcommittee on Review of the GRAS List (Phase II). 1972. A comprehensive survey of industry on the use of food chemicals generally recognized as safe (GRAS). Prepared under DHEW contract no. FDA 70-22 by Committee on Food Protection, Division of Biology and Agriculture, National Research Council, Washington, D.C.

18. U.S. Bureau of the Census, Census of Agriculture, 1969. Mustard seed. Page 56 in vol. II. General report, chapter 6, Crops, nursery and greenhouse products, forest products. U.S. Government Printing Office, Washington, D.C. 1973.

19. U.S. Bureau of the Census, Foreign Trade Division. 1965-1972. [Mustard, ground or prepared, TSUSA no. 1615900; mustard seeds, whole, TSUSA no. 1616100] U.S. foreign trade imports TSUSA commodity by country. Annual. Rept. FT 246 rev. Government Printing Office, Washington, D.C.

BEST ORIGINAL COPY

20. Nelson, A.A. 1954. Approximate relation of parts per million in diet to mg/kg/day (table). Assoc. Food Drug Officials Quart. Bull. 18:66.

21. Altman, P.L., and D.S. Dittmer, editors. 1972. Pages 201-202 in Biology data book, 2nd ed., vol. 1. Federation of American Societies for Experimental Biology, Bethesda, Maryland.

22. Jenner, P.M., E.C. Hagan, J.M. Taylor, E.L. Cook, and O.G. Fitzhugh. 1964. Food flavourings and compounds of related structure. I. Acute oral toxicity. Food Cosmet. Toxicol. 2:327-343.

23. Klesse, P., and P. Lukoschek. 1955. Untersuchungen über die bakteriostatische wirksamkeit einiger senföle. Arzn. Forsch. 5:505-507. (In German; translation supplied with reference no. 1).

24. Smyth, H.F., Jr., C.P. Carpenter, C.S. Weil, U.C. Pozzani, J.A. Striegel, and J.S. Nycum. 1969. Range-finding toxicity data: list VII. Amer. Ind. Hyg. Assoc. J. 30:470-478.

25. Lewin, L. 1879. Ueber das verhalten der xanthogen säure und der xanthogensauren alkalien im thierischen organismus und die giftwirkung des schwefelkohlenstoffs. Virchows Arch. Pathol. Anat. Physiol. Klin. Med. 78:113-138.

26. Samochowiec, L., J. Wojcicki, and K. Dominiczak. 1967. Der einfluss der "essentiellen" phospholipide auf leberveränderungen nach chronischer äthylalkohol-und allylisosulfocyanat-intoxikation bei weissen ratten. Arzn. Forsch. 17:1374-1376. (In German; translation supplied with reference no. 1).

27. Ahmad, K., F.M.M. Rahman, A. Rahman, and R. Begum. 1967. XIII. Pharmacological and toxicological effects of food components. Biochemical effect of allylisothiocyanate. Proc. 7th. Int. Congr. Nutr. 5:815-819.

28. Hagan, E.C., W.H. Hansen, O.G. Fitzhugh, P.M. Jenner, W.I. Jones, J.M. Taylor, E.L. Long, A.A. Nelson, and J.B. Brouwer. 1967. Food flavourings and compounds of related structure. II. Subacute and chronic toxicity. Food Cosmet. Toxicol. 5:141-157.

BEST ORIGINAL COPY

29. Food and Drug Research Laboratories, Inc. 1973. Teratologic evaluation of FDA 71-26 (oil of mustard) in mice, rats, hamsters, and rabbits. Final report under contract no. FDA 71-260. Maspeth, N.Y. [55 pp.]

30. Reid, B.L. [March 24, 1975.] Mustard oil (allyl isothiocyanate). Toxicity and teratogenicity studies in avian embryos. Report prepared under DHEW contract no. FDA 72-345. Department of Poultry Science, University of Arizona, Tucson, Arizona. [17 pp.]

31. Litton Bionetics, Inc. 1975. Summary of mutagenicity, screening studies, host-mediated assay, cytogenetics, dominant lethal assay contract FDA 71-268, compound FDA 71-26, oil of mustard. Kensington, Md. 155 pp.

32. Rusch, H.P., D. Bosch, and R.K. Boutwell. 1955. The influence of irritants on mitotic activity and tumor formation in mouse epidermis. Acta Unio Int. Contra Cancrum 11:699-703.

33. Visser, J., and R.E.J. Ten Seldam. 1938. Over de "carcinogene" en anticarcinogene eigenschappen van verschillende huidirritantia. Geneesk. Tijdschr. Ned. Indie 78:3280-3291.

34. Soboleva, N.G. 1927. Deistvuyushchii nachalo gorchitsy kak khronicheskii razdrazhitel' kozhi u myshei. (In Russian) Vestn. Rentgenol. Radiol. 5:191-198.

35. Carratala, R., and D. Lambre. 1951. Sulfocarbonismo: estudio experimental. Rev. Asoc. Med. Argent. 65:282-284.

36. Blair, J. 1965. Coronary disease associated with ingestion of mustard. Ohio State Med. J. 61:732-734.

37. Perry, T.L., S. Hansen, M. Hestrin, and L. MacIntyre. 1965. Exogenous urinary amines of plant origin. Clin. Chem. Acta 11:24-34.

38. Larsen, P.O. 1965. Occurrence of p-hydroxybenzylamine in white mustard (Sinapis alba L.). Biochim. Biophys. Acta 107: 134-136.

39. Slaughter, J.C., and A.R.A. Uvgard. 1972. The identification of p-hydroxybenzylamine in barley and malt. Phytochemistry 11:478-479.

BEST ORIGINAL COPY

40. Skramlik, E. V. 1959. Uber die giftigkeit und verträglichkeit von ätherischen ölen. Pharmazie 14:435-445. (In German; translation supplied with reference no. 1).

41. Jalil, M. A., and M. S. Rahman. 1969. The effect of certain dietary vegetable oils and cholesterol on lipid pattern in serum and liver of rats. Pak. J. Biochem. 2:41-46.

42. Brahmachari, B. B. 1934. Nutritive value of mustard oil. Indian Med. Gaz. 69:327-329.

43. Bandyopadhyay, A., and S. Banerjee. 1963. Dietary fats and plasma lipids in chicks. Proc. Soc. Exp. Biol. Med. 114:222-224.

44. Banerjee, S., P. N. Rao, and S. K. Ghosh. 1965. Biochemical and histochemical changes in aorta of chicks fed vegetable oils and cholesterol. Proc. Soc. Exp. Biol. Med. 119:1081-1086.

45. Glatzel, H. 1965. Spices and organ functions. Results of recent studies. Muenchen. Med. Wochenschr. 107:332-342. (In German).

46. Bandyopadhyay, A., and S. Banerjee. 1964. Effect of vegetable oils on tissue lipids of rhesus monkeys. Indian J. Exp. Biol. 2:16-19.

47. Banerjee, S., P. K. Ghosh, and A. Bandyopadhyay. 1962. Effect of vegetable oils on plasma lipids of rhesus monkeys. Proc. Soc. Exp. Biol. Med. 109:313-317.

48. Gupta, K. K., and R. M. Rai. 1963. Effect of fats used in Indian diet on cholesterol and blood coagulation. J. Assoc. Physicians India. 11:213-220.

49. Ranadive, K. J., and S. V. Gothoskar. 1965. Testing carcinogenicity of edible oils. Part II. Mustard oil. Indian J. Med. Res. 53:980-983.

50. Ranadive, K. J., S. V. Gothoskar, and B. V. Tezabwala. 1972. Carcinogenicity of contaminants in indigenous edible oils. Int. J. Cancer 10:652-666.

51. Ranadive, K. J., S. V. Gothoskar, and B. V. Tezabwala. 1973. Testing carcinogenicity of contaminants in edible oils. Argemone oil in mustard oil. Indian J. Med. Res. 61:428-434.

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NATIONAL TOXICOLOGY PROGRAM
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No. 234

CARCINOGENESIS BIOASSAY OF ALLYL ISOTHIOCYANATE

(CAS NO. 57-06-7)

IN F344/N RATS AND B6C3F$_1$ MICE (GAVAGE STUDY)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

NATIONAL TOXICOLOGY PROGRAM

The National Toxicology Program (NTP), established in 1978, develops and evaluates scientific information about potentially toxic and hazardous chemicals. This knowledge can be used for protecting the health of the American people and for the primary prevention of chemically induced disease. By bringing together the relevant programs, staff, and resources from the U.S. Public Health Service, DHHS, the National Toxicology Program has centralized and strengthened activities relating to toxicology research, testing and test development/validation efforts, and the dissemination of toxicological information to the public and scientific communities and to the research and regulatory agencies.

The NTP is comprised of four charter DHHS agencies: the National Cancer Institute, National Institutes of Health; the National Institute of Environmental Health Sciences, National Institutes of Health; the National Center for Toxicological Research, Food and Drug Administration; and the National Institute for Occupational Safety and Health, Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS.

NTP TECHNICAL REPORT
ON THE

CARCINOGENESIS BIOASSAY OF ALLYL ISOTHIOCYANATE

(CAS NO. 57-06-7)

IN F344/N RATS AND B6C3F$_1$ MICE (GAVAGE STUDY)

NATIONAL TOXICOLOGY PROGRAM
Box 12233
Research Triangle Park
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Bethesda, Maryland 20205

October 1982

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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

NOTE TO THE READER

This is one in a series of experiments designed to determine whether selected chemicals produce cancer in animals. Chemicals selected for testing in the NTP carcinogenesis bioassay program 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 carcinogenic potential. Negative results, in which the test animals do not have a greater incidence of cancer than control animals, do not necessarily mean that a test chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a test chemical is carcinogenic for animals under the conditions of the test and indicate that exposure to the chemical is a potential hazard to humans. The determination of the risk to humans from chemicals found to be carcinogenic in animals requires a wider analysis which extends beyond the purview of this study.

This study was initiated by the National Cancer Institute's Carcinogenesis Testing Program, now part of the National Institute of Environmental Health Sciences, National Toxicology Program.

Comments and questions about the National Toxicology Program Technical Reports on Carcinogenesis Bioassays should be directed to the National Toxicology Program, located at Room A-306, Landow Building, Bethesda, MD 20205 (301-496-1152) or at Research Triangle Park, NC 27709 (919-541-3991).

Although every effort is made to prepare the Technical Reports as accurately as possible, mistakes may occur. Readers are requested to communicate any mistakes to the Deputy Director, NTP (P.O. Box 12233, Research Triangle Park, NC 27709), so that corrective action may be taken. Further, anyone who is aware of related ongoing or published studies not mentioned in this report is encouraged to make this information known to the NTP.

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CARCINOGENESIS BIOASSAY OF ALLYL ISOTHIOCYANATE

$$CH_2=CH-CH_2-N=C=S$$

ALLYL ISOTHIOCYANATE

CAS NO. 57-06-7
C_4H_5NS Mol. Wt. 99.16

ABSTRACT

A 2-year carcinogenesis bioassay of food-grade allyl isothiocyanate (greater than 93% purity), a flavoring agent, was conducted by administering 12 or 25 mg/kg allyl isothiocyanate in corn oil five times per week by gavage to groups of 50 F344/N rats and 50 B6C3F1 mice of each sex for 103 weeks. Groups of 50 rats and 50 mice of each sex received corn oil alone and served as vehicle controls.

A single-dose study, a 14-day study, and a 13-week study were performed before the chronic study was conducted. Pathologic findings seen in the 14-day study at 50 mg/kg included a thickened mucosal surface of the stomach in rats and mice and a thickened urinary bladder wall in male mice. No gross or microscopic lesions were seen at the highest dose level (25 mg/kg) in the 13-week study.

In the chronic study, survival of dosed and control rats of each sex was comparable. Throughout the study, the mean body weights of high-dose male rats were lower than those of the controls, while during the last half of the study the mean body weights of the low-dose and high-dose female rats were higher than the mean body weights of the control animals. Final body weights in control and dosed groups were comparable.

Transitional-cell papillomas in the urinary bladder occurred in dosed male rats with a statistically significant trend ($P<0.05$; controls, 0/49, 0%; low-dose, 2/49, 4%; high-dose, 4/49, 8%). This tumor has not been observed among 568 untreated male control F344/N rats at this laboratory. The incidence of transitional-cell papillomas in male vehicle control rats in all laboratories in the NCI/NTP Bioassay Program is 1/994 (0.1%). Epithelial hyperplasia in the urinary bladder was also observed at increased incidences in dosed male rats (0/49, 1/49, 6/49). The hyperplasia did not occur in the same animals that had papillomas.

Fibrosarcomas in the subcutaneous tissue occurred in female rats with a statistically significant positive trend ($P<0.05$; controls, 0/50, 0%; low-dose, 0/50, 0%; high-dose, 3/50, 6%), but the incidence in the high-dose group was not significant when compared with that in the control group. The historical incidence of this lesion is 1/591 (0.2%) in untreated control female F344/N rats at this laboratory and 9/999 (0.9%) in female gavage control rats in all laboratories in the Bioassay Program.

Survival of control and dosed female mice, although comparable, was unusually low. Mean body weights of high-dose mice of each sex were higher than those of the controls throughout most of the study. Final body weights in control and dosed groups were comparable. The mice probably did not receive the maximum tolerated dose of allyl isothiocyanate.

The increased incidence of cytoplasmic vacuolization in the liver of dosed male mice was related to administration of allyl isothiocyanate (controls, 2/49, 4%; low-dose, 8/49, 16%; high-dose, 13/50, 26%).

Under the conditions of this bioassay, allyl isothiocyanate was carcinogenic for male F344/N rats, causing transitional-cell papillomas in the urinary bladder. Evidence for associating allyl isothiocyanate with subcutaneous fibrosarcomas in female F344/N rats was equivocal. Allyl isothiocyanate was not carcinogenic for B6C3F1 mice of either sex.

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The bioassay of allyl isothiocyanate was conducted at Southern Research Institute under a subcontract to Tracor Jitco, Inc., the prime contractor for the Carcinogenesis Testing Program. The chronic study was begun in March 1978 and completed in April 1980.

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*Unable to attend June 23, 1981 meeting

SUMMARY OF PEER REVIEW COMMENTS ON THE BIOASSAY OF ALLYL ISOTHIOCYANATE

On June 23, 1981, this carcinogenesis bioassay report on allyl isothiocyanate underwent peer review and was approved by the National Toxicology Program Board of Scientific Counselors' Technical Reports Review Subcommittee and associated Panel of Experts at an open meeting held in Building 101, National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. Williams, as a principal reviewer for the report on the bioassay of allyl isothiocyanate, agreed with the conclusions that, under the conditions of the bioassay, allyl isothiocyanate was carcinogenic to male F344/N rats, causing transitional-cell papillomas in the urinary bladder. Evidence for associating allyl isothiocyanate with subcutaneous fibrosarcomas in female rats was equivocal. The chemical was not carcinogenic for B6C3F1 mice of either sex. He stated that the discussion should emphasize that this compound was associated with only a low incidence of benign bladder tumors under conditions of exposure that are known to affect the physiology of urine excretion.

As the second principal reviewer, Dr. Hitchcock said there was quite low survival in control and high-dose female mice and suggested that some explanation should have been given for this. She noted the incidence of eye lesions which may have been due to groups of rats being housed near the light source without rotation of cages. Dr. Shore asked whether attention could be given to balancing cage position in the room. Dr. G. Boorman, NTP, replied that one problem with cage rotation is that it may enhance the chances for gavage errors; he further stated that the NTP was investigating this recurring phenomenon and would consider the option of cage rotation as well as reduced light intensity. Dr. Hitchcock asked that recent negative results with *Salmonella* be mentioned. Dr. Swenberg said that the discussion should include comment that allyl isothiocyanate may possibly be working as a tumor promoter.

Dr. Williams moved that the report on the bioassay of allyl isothiocyanate be accepted. Dr. Hitchcock accepted the motion, and the report was approved unanimously by the Peer Review Panel.

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I. INTRODUCTION

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$$CH_2=CH-CH_2-N=C=S$$

ALLYL ISOTHIOCYANATE

CAS NO. 57-06-7
C_4H_5NS Mol. Wt. 99.16

Allyl isothiocyanate is the major component in volatile oil of mustard, a flavoring agent prepared from seeds of black mustard (*Brassica nigra*) (Life Sciences Research Office, 1975). Synthetically prepared allyl isothiocyanate and volatile oil of mustard are approved by the U.S. Food and Drug Administration for use as flavoring agents (U.S. CFR, 1979); the Food Chemicals Codex (1972) specifies that the oil should contain not less than 93% allyl isothiocyanate. Allyl isothiocyanate is also found in cabbage, broccoli, kale, cauliflower, and horseradish (Mitchell and Jordan, 1974; Life Sciences Research Office, 1975; Hall, 1973).

Volatile oil of mustard is used in pickling spices and imitation pineapple flavoring (Kirk-Othmer, 1966). Allyl isothiocyanate may be present in the following foods: syrups (10-88 ppm), meats (87 ppm), condiments (52 ppm), baked goods (5.2 ppm), candy, ice cream, and ices (0.50 ppm), and nonalcoholic beverages (0.02-0.50 ppm) (Life Sciences Research Office, 1975). Allyl isothiocyanate is also used as a denaturant for alcohol and as a medicinal counter-irritant (Merck Index, 1976; Kirk-Othmer, 1965).

Approximately 33,000 pounds of allyl isothiocyanate were used by the food industry in the United States in 1970 (Life Sciences Research Office, 1975). The amount of synthetic allyl isothiocyanate produced in 1979 exceeded 1,000 pounds, but specific production figures are not available (USITC, 1979). Thirty-two thousand metric tons of mustard seed were imported into the United States in 1978 (Kirk-Othmer, 1980).

The oral LD_{50} value of allyl isothiocyanate is reported to be 339 mg/kg for Osborne-Mendel rats (Jenner et al., 1964) and 490 mg/kg for male rats of an unspecified strain (Vernot et al., 1977). The subcutaneous LD_{50} value for white mice is 80 mg/kg (Klesse and Lukoschek, 1955).

Administration of allyl isothiocyanate has been shown to affect various functions and organs in the rat. Radioiodine uptake by the thyroid was depressed and the relative weight of the thyroid was increased in male Wistar rats administered 2-to 5-mg doses of allyl isothiocyanate by gavage daily for 1 to 60 days (Langer and Greer, 1968; Langer and Stole, 1965). Hyperplastic areas were observed in the thyroid of female Holtzman rats 12 days after they received two 100 mg/kg subcutaneous doses of allyl isothiocyanate (Nishie and Daxenbichler, 1980). The blood coagulation time for male Sprague-Dawley rats given daily 0.5 mg intraperitoneal injections of allyl isothiocyanate for 30 days was 60% of the value for controls (Muztar et al., 1979b). A twofold increase in urine volume, an increase in the total amount of uric acid, creatinine, and glucose excreted during a 24-hour period, and an increase in the concentration of uric acid in the urine compared with that of controls were observed in male Sprague-Dawley rats fed diets containing 100 or 300 ppm allyl isothiocyanate (Muztar et al., 1979a; Muztar et al., 1979b).

Epithelial hyperplasia of the nonglandular portion of the stomach, with acute to subacute ulcers 2 to 6.5 mm in diameter, was observed in all Osborne-Mendel rats of either sex administered 50 mg/kg allyl isothiocyanate by gavage for 20 days and in 50% of the rats receiving 20 mg/kg. Minor inflammatory foci were observed in the liver of rats receiving the higher dose (Hagan et al., 1967).

Allyl isothiocyanate was not mutagenic in *Bacillus subtilis* H17 and M45, *Escherichia coli* WP2, or *Salmonella typhimurium* TA 98, 100, 1535, or 1537 (with or without metabolic activation) (Oda et al., 1978; Eder et al., 1980; NTP, 1981). Allyl isothiocyanate was fetotoxic for Holtzman rats (Nishie and Daxenbichler, 1980),

but was not found to be teratogenic in Wistar rats (Ruddick et al., 1976).

The Food and Drug Administration has prepared three reviews on oil of mustard (90% allyl isothiocyanate), a food additive generally recognized as safe (NTIS, 1972; NTIS, 1973; NTIS, 1975). These reviews emphasize the lack of data on the carcinogenicity and toxicity of these substances. The FDA cites some evidence for increased fetal deaths and resorptions in rodents when oil of mustard is administered at 28.0 mg/kg for 10 consecutive days (from days 6 to 15 of gestation) to pregnant mice (albino CD-1 outbred mice). Other teratology studies in rats, hamsters, and rabbits were considered negative (NTIS, 1973). A select committee of the Federation of American Societies for Experimental Biology (FASEB) stated that "more definitive toxicological studies" on oil of mustard were warranted. Using the data available in 1975, FASEB concluded that there was no indication that allyl isothiocyanate was a hazard to the public at levels currently used in food (NTIS, 1973).

The NCI/NTP Bioassay Program tested allyl isothiocyanate because it is a widely used food additive that had not been tested for carcinogenicity.

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CHEMICAL ANALYSIS

Food-grade allyl isothiocyanate (CAS No. 57-06-7), greater than 93% allyl isothiocyanate, was obtained from Arsynco, Inc. (Carestadt, NJ) in a single batch (Lot No. 532251).

The results of the analyses performed at Midwest Research Institute (Appendix E) indicated the following: elemental analyses agreed with theoretical values; gas-liquid chromatography on two different systems detected at least six minor impurities with areas totaling less than 1% of the major peak; thin-layer chromatography in two systems detected only one spot; the infrared and ultraviolet spectra were consistent with the structure and spectra reported in the literature (Sadtler Research Laboratories); and the nuclear magnetic resonance spectrum was consistent with the structure. The nuclear magnetic resonance spectrum indicated the presence of a minor impurity that could be the thiocyanate. The identity of this minor impurity was not pursued.

Southern Research Institute analyzed the chemical periodically throughout the study by gas-liquid chromatography and infrared spectroscopy. The results indicated no breakdown of the bulk material during the study.

DOSAGE PREPARATION

Dosage mixtures of allyl isothiocyanate were prepared daily in the single-dose and 14-day studies and were prepared weekly in the 13-week and chronic studies. Mixtures were obtained by pipetting the appropriate amount of the chemical in a beaker and dissolving it in a small amount of corn oil. This stock solution was diluted with additional corn oil to the desired final volume. Concentrations of the test substance were based on the volume of the chemical in relation to the volume of corn oil.

Analysis of the stability of allyl isothiocyanate in corn oil was performed at Midwest Research Institute by assaying samples of corn oil mixtures containing 0.05% test chemical that had been stored at room temperature for 7 days (Appendix F). The corn oil/allyl isothiocyanate solutions were then diluted with anhydrous ethyl ether, and the concentration of the test chemical was determined by vapor-phase chromatography. Allyl isothiocyanate was found to be stable in corn oil for 7 days at room temperature with a recovery of 99.5%. Selected batches of corn oil gavage mixtures administered during the chronic study were analyzed at Southern Research Institute to determine the adequacy of preparation; differences between the mean sample concentration and the targeted concentration were 0.01% (v/v) or less (Table G1).

Four samples of corn oil gavage mixtures prepared and analyzed at Southern Research Institute were shipped to either Midwest Research Institute or Raltech Scientific Services, Inc., for referee analysis of allyl isothiocyanate. The results from the three laboratories were in agreement.

PRECHRONIC STUDIES

Single-Dose Study

Groups of five F344/N rats of each sex were administered a single dose of allyl isothiocyanate (25, 50, 100, 200, or 400 mg/kg body weight) in corn oil by gavage. Groups of five B6C3F1 mice of each sex received 50, 100, 200, 400, or 800 mg/kg allyl isothiocyanate by the same route. No controls were used.

Animals were observed twice daily for 16 days. Weights were taken on the day of dosing and then on day 15. The peritoneal cavities were examined in male mice administered 200, 400, or 800 mg/kg and in female mice administered 100, 200, or 400 mg/kg.

Further details of the study are presented in Table 1.

Fourteen-Day Study

Groups of five F344/N rats of either sex were administered 25, 50, 100, 200, or 400 mg/kg allyl isothiocyanate in corn oil by gavage for 14 consecutive days (Table 1). Groups of B6C3F1 mice received 3, 6, 12, 25, or 50 mg/kg by the same route. No controls were used.

Rats and mice were observed twice daily and were weighed on days 1 and 15 of the study. Gross necropsies were performed on all animals.

Thirteen-Week Study

Thirteen-week studies were conducted to evaluate the cumulative toxicity of allyl isothiocyanate and to determine the doses to be used in the chronic studies.

Groups of 10 rats and mice of each sex received 1.5, 3, 6, 12, or 25 mg/kg allyl isothiocyanate by gavage 5 days per week for 13 weeks (Table 1). Vehicle controls received corn oil alone.

All animals were checked for mortality and clinical signs of toxicity and morbidity twice daily. Moribund animals were killed and necropsied. Individual animals were weighed weekly.

From days 92 to 96, survivors were killed with carbon dioxide. Necropsies were performed on animals that survived to day 92 and on all animals found dead, unless precluded in whole or part by autolysis or cannibalism. The following specimens were examined histologically in vehicle-control and high-dose groups: gross lesions, tissue masses, abnormal lymph nodes, skin, mandibular lymph nodes, mammary gland, salivary gland, thigh muscle, bone marrow, trachea, lungs and bronchi, heart, thyroid, parathyroid, esophagus, stomach, cecum, duodenum, jejunum, ileum, colon, mesenteric lymph nodes, liver, gallbladder (mice), pancreas, spleen, thymus, kidneys, adrenals, urinary bladder, seminal vesicles/prostate/testes or ovaries/uterus, brain, and pituitary. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

CHRONIC STUDY

Study Design

Groups of 50 rats and 50 mice of each sex received 12 or 25 mg/kg allyl isothiocyanate in corn oil by gavage 5 times per week (Monday through Friday) for 103 weeks (Table 1). Groups of 50 rats and 50 mice of each sex received corn oil on the same schedule and served as vehicle controls.

Control and dosed groups were of the same strain, sex, and age range and were from the same source and shipment. All animals were housed in the same room, and no other chemicals were on test in that room. Neither cages nor racks were rotated. The animal cages were housed on two racks, each rack having six levels. On one rack, high-dose males were on the top two levels, high-dose females were on the middle two levels, and low-dose males were on the bottom two levels. On the other rack, low-dose females were placed on the top two levels, control males were on the middle two levels, and control females were on the bottom two levels. All aspects of animal care and maintenance were similar. Animals were randomized to control and dosed groups as described in Table 1. Chronic studies for rats and mice began in March 1978.

Clinical Examinations and Pathology

All animals were observed twice daily for signs of morbidity and mortality. Clinical signs and body weights by cage were recorded every 4 weeks. The mean body weight of each group was calculated by dividing the total weight of all animals in the group by the number of surviving animals in the group. Moribund animals and animals that survived to the end of the bioassay were killed with carbon dioxide and necropsied.

Examinations for grossly visible lesions were performed on major tissues or organs. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The following were examined microscopically: tissue masses, abnormal lymph nodes, skin, mandibular lymph nodes, mammary gland, salivary gland, thigh muscle, bone marrow, femur, thymus, trachea, lungs and bronchi, heart, thyroid, parathyroid, esophagus, stomach, duodenum, jejunum, ileum, colon, mesenteric lymph nodes, liver, gallbladder (mice), pancreas, spleen, kidneys, adrenals, urinary bladder, seminal vesicles/prostate/testes or ovaries/ uterus, brain, and pituitary. Oil Red O on frozen sections was used to more clearly

define the nature of cytoplasmic vacuolization in the livers of male mice.

Necropsies were performed on all animals found dead and on those killed at the end of the study, unless precluded in whole or in part by autolysis or cannibalism. Thus, the number of animals from which particular organs or tissues were examined microscopically varies and is not necessarily equal to the number of animals that were placed on study in each group.

The pathology report and selected slides were evaluated by the NTP Pathology Working Group as described by Ward et al. (1978). The diagnoses represent a consensus of contracting pathologists and the NTP Pathology Working Group.

Data Recording and Statistical Methods

Data on this experiment were recorded in the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, clinical observations, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969).

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and Tarone's (1975) extensions of Cox's methods for testing for a dose-related trend.

The incidence of neoplastic or nonneoplastic lesions has been given as the ratio of the number of animals bearing such lesions at a specific anatomic site to the number of animals in which that site was examined. In most instances, the denominators included only those animals for which that site was examined histologically. However, when macroscopic examination was required to detect lesions (e.g., skin or mammary tumors) prior to histologic sampling, or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the numbers of animals necropsied.

For the statistical analysis of tumor incidence data, two different methods of adjusting for intercurrent mortality were employed. Each used the classical methods for combining contingency tables developed by Mantel and Haenszel (1959). Tests of significance included pairwise comparisons of high-and low-dose groups with controls and tests for overall dose-response trends.

The first method of analysis assumed that all tumors of a given type observed in animals dying before the end of the study were "fatal"; i.e., they either directly or indirectly caused the death of the animal. According to this approach, the proportions of tumor-bearing animals in the dosed and control groups were compared at each point in time at which an animal died with a tumor of interest. The denominators of these proportions were the total number of animals at risk in each group. These results, including the data from animals killed at the end of the study, were then combined by the Mantel-Haenszel methods to obtain an overall P-value. This method of adjusting for intercurrent mortality is the life table method of Cox (1972) and of Tarone (1975).

The second method of analysis assumed that all tumors of a given type observed in animals dying before the end of the study were "incidental"; i.e., they were merely observed at autopsy in animals dying of an unrelated cause. According to this approach, the proportions of animals found to have tumors in dosed and control groups were compared in each of five time intervals: 0-52 weeks, 53-78 weeks, 79-92 weeks, week 93 to the week before terminal kill, and the terminal kill period. The denominators of these proportions were the number of animals actually autopsied during the time interval. The individual time interval comparisons were then combined by the previously described methods to obtain a single overall result. (See Peto et al., 1980, for the computational details of both methods.)

In addition to these tests, one other set of statistical analyses was carried out and reported in the tables analyzing primary tumors; the Fisher's exact test for pairwise comparisons and the Cochran-Armitage linear trend test for dose-response trends (Armitage, 1971; Gart et al., 1979). The tests were based on the overall proportion of tumor-bearing animals. All reported P values are one-sided. For studies in which there is little effect of compound administration on survival, the results of the three alternative analyses will generally be similar. When differing results are obtained by the three methods, the final interpretation of the data will depend on the extent to which the tumor under consideration is regarded as being the cause of death.

TABLE 1. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS

	Single-Dose Study	14-Day Study	13-Week Study	Chronic Study
Experimental Design				
Size of Test Groups	5 males, 5 females of each species	5 males, 5 females of each species	10 males, 10 females of each species	50 males, 50 females of each species
Doses	Rats: 25, 50, 100, 200, or 400 mg/kg body weight allyl isothiocyanate in corn oil; volume: 10 ml/kg body weight Mice: 50, 100, 200, 400, or 800 mg/kg body weight allyl isothiocyanate in corn oil; volume: 10 ml/kg body weight.	Rats: 25, 50, 100, 200, or 400 mg/kg body weight allyl isothiocyanate in corn oil; volume: 10 ml/kg body weight Mice: 3, 6, 12, 25, or 50 mg/kg body weight allyl isothiocyanate in corn oil; volume: 10 ml/kg body weight	Rats and mice: 1.5, 3, 6, 12, or 25 mg/kg body weight allyl isothiocyanate in corn oil; vehicle control, corn oil only, volume: rats, 5 ml/kg body weight; mice, 10 ml/kg body weight	Rats and mice: low dose 12 mg/kg body weight allyl isothiocyanate in corn oil; high dose 25 mg/kg body weight allyl isothiocyanate in corn oil; vehicle control: corn oil; volume: rats, 5 ml/kg body weight; mice, 10 ml/kg body weight
Duration of Dosing	Rats and mice: single dose; killed on day 16	Rats: 14 consecutive days; killed on days 16-17 Mice: 14 consecutive days; killed on days 17-31	Rats and mice: 13 weeks, 5 days per week; killed on days 92-96	Rats and mice: 103 weeks; 5 days per week; killed at week 104-106
Type and Frequency of Observation	Observed twice daily for mortality	Observed twice daily for mortality	Observed twice daily for morbidity and mortality	Observed twice daily for morbidity and mortality
Necropsy and Histologic Examination	Peritoneal cavity examined in male mice receiving 200, 400, or 800 mg/kg and in female mice receiving 100, 200, or 400 mg/kg	All animals necropsied	Gross necropsy performed on all animals; histologic examination performed on all vehicle controls and all animals receiving 25 mg/kg	Gross necropsy and histologic examination performed on all animals

TABLE 1. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS (Continued)

	Single-Dose Study	14-Day Study	13-Week Study	Chronic Study
Animals and Animal Maintenance				
Species	F344/N Rats; B6C3F1 Mice	F344/N Rats; B6C3F1 Mice	F344/N Rats; B6C3F1 Mice	F344/N Rats; B6C3F1 Mice
Animal Source	Frederick Cancer Research Center (Frederick, MD)	Same as single-dose study	Same as single-dose study	Harlan Industries, Inc. (Indianapolis, IN)
Time Held Before Start of Test	Rats: 9 days Mice: 8 days	Rats: 8 days Mice: 8 days	Rats: 5 days Mice: 5 days	Rats: 16 days Mice: 16 days
Age When Placed on Study	35 days old	35 days old	35 days old	Rats: 39 days old Mice: 57 days old
Age When Killed	51 days old	Rats: 51-52 days old Mice: 52-66 days old	127-131 days old	Rats: 767 days old Mice: 785 days old
Method of Animal Distribution	Randomized to cages using table of random numbers; cages randomized to test groups using another table of random numbers	Same as single-dose study	Same as single-dose study	Same as single-dose study
Feed	Wayne Lab Blox® Allied Mills, Inc. (Chicago, IL) Available *ad libitum*	Same as single-dose study	Same as single-dose study	Same as single-dose study
Bedding	Beta Chips®, hardwood chips, Northeastern Products Corp. (Warrensburg, NY)	Same as single-dose study	Same as single-dose study	Same as single-dose study
Water	Tap water in glass bottles available *ad libitum*	Same as single-dose study	Tap water via automatic system, Edstrom Industries, Inc. (Waterford, WI)	Same as 13-week study
Cages	Stainless steel, Hahn Roofing and Sheet Metal Co. (Birmingham, AL)	Same as single-dose study	Polycarbonate Lab Products, Inc. (Garfield, NJ)	Same as 13-week study

TABLE 1. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS (Continued)

	Single-Dose Study	14-Day Study	13-Week Study	Chronic Study
Animals and Animal Maintenance				
Animals Per Cage	5	5	5	5
Cage Filters	Fiberglass	Fiberglass	Disposable spun-bonded Polyester Dupont #2024 Snow Filtration Co. (Cincinnati, OH)	Same as 13-week study
Animal Room Environment	23° ± 3°C; humidity uncontrolled; 15 air changes per hr. 9 hrs fluorescent light	Same as single-dose study	23°±3°C; humidity uncontrolled; 15 air changes per hr. 12 hrs fluorescent light	23°±3°C; humidity uncontrolled; 15 air changes per hr. 12 hrs fluorescent light
Other Chemicals on Test in Same Room	Rats and mice: ethyl acrylate, eugenol, D-mannitol:	Rats: ethyl acrylate, eugenol, D-mannitol: Mice: ethyl acrylate, eugenol, D-mannitol; stannous chloride, ziram, propyl gallate, zearalenone	None	None
Chemical/Vehicle Mixture				
Preparation	Allyl isothiocyanate mixed with Mazola® corn oil to concentration of highest dose (stock mixture); stock mixture diluted with corn oil to make other doses	Same as single-dose study	Same as single-dose study	Same as single-dose study
Frequency of Preparation	Mixture prepared daily	Mixture prepared daily	Mixture prepared once each week	Mixture prepared once each week
Storage Conditions		Excess mixture discarded		Dosing mixture stored at 5°C for no longer than 10 days

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III. RESULTS

RATS

PRECHRONIC STUDIES

Single-Dose Study

Fourteen-Day Study

Thirteen-Week Study

CHRONIC STUDY

Body Weights and Clinical Signs

Survival

Pathology and Statistical Analyses of Results

MICE

PRECHRONIC STUDIES

Single-Dose Study

Fourteen-Day Study

Thirteen-Week Study

CHRONIC STUDY

Body Weights and Clinical Signs

Survival

Pathology and Statistical Analyses of Results

PRECHRONIC STUDIES

Single-Dose Study

All animals survived to the end of the 16-day observation period. The following average weight increases over the initial weight (on day 0) were measured:

Dose (mg/kg)	Weight Increase (Percent)	
	Males	Females
25	69	40
50	58	45
100	61	44
200	50	38
400	31	20

Other signs of toxicity seen in male rats receiving 200-400 mg/kg included inactivity, watery eyes, and ruffled fur. All signs were gone by day 9 in the 400 mg/kg group and by day 3 in the 200 mg/kg group. Female rats also exhibited inactivity and ruffled fur. Since no rats died during the course of those studies, the highest dose for the 14-day study was set at 400 mg/kg.

Fourteen-Day Study

All rats administered 200 or 400 mg/kg allyl isothiocyanate died before the end of the study (Table 2). Animals administered 100 mg/kg gained less weight than did animals receiving lower doses. A thickened mucosal surface of the stomach was seen in groups of males and females administered 50-400 mg/kg, and adhesion of the stomach to the peritoneum was observed in groups of male rats receiving 50-400 mg/kg and in groups of female rats receiving 100-400 mg/kg (Table 3).

Toxic signs were seen at all dose levels. These signs included inactivity and ruffled fur and were most severe at the 400 mg/kg dose level. Due to the toxicity and pathologic effects observed, the highest dose for the 13-week study was set at 25 mg/kg.

TABLE 2. DOSAGE, SURVIVAL, AND MEAN BODY WEIGHTS OF RATS RECEIVING ALLYL ISOTHIOCYANATE BY GAVAGE FOR 14 DAYS

Dose (mg/kg)	Survival *(a)*	Mean Body Weight (grams)		
		Initial	Final	Change *(b)*
Males				
25	5/5	96.6 ± 5.0	147.0 ± 6.6	+50.4 ± 2.8
50	5/5	85.8 ± 3.9	127.2 ± 4.1	+41.4 ± 2.3
100	5/5	92.8 ± 7.1	113.0 ± 6.1	+20.2 ± 2.2
200	0/5*(c)*	*(d)*	*(d)*	*(d)*
400	0/5*(e)*	*(d)*	*(d)*	*(d)*
Females				
25	5/5	82.6 ± 2.7	113.2 ± 1.7	+30.6 ± 2.3
50	5/5	77.4 ± 3.5	105.6 ± 3.2	+28.2 ± 2.6
100	5/5	84.8 ± 3.0	105.8 ± 3.8	+21.0 ± 2.7
200	0/5*(f)*	*(d)*	*(d)*	*(d)*
400	0/5*(g)*	*(d)*	*(d)*	*(d)*

(a) Number surviving/number initially in the group. All calculations refer only to the survivors of each group.
(b) Mean weight change of the survivors of the group ± standard error of the mean.
(c) Days of death: 2, 2, 3, 8, 9
(d) No data are presented due to the 100% mortality in this group.
(e) Days of death: 2, 2, 2, 2, 4
(f) Days of death: 2, 2, 6, 8, 9
(g) Days of death: 2, 2, 2, 2, 3

TABLE 3. INCIDENCE OF COMPOUND-RELATED EFFECTS OBSERVED IN RATS AT NECROPSY IN THE 14-DAY STUDY OF ALLYL ISOTHIOCYANATE

Dose (mg/kg)	Thickened Mucosal Surface of Stomach	Adhesion of Stomach to Peritoneum
Males		
25	0/5	0/5
50	5/5	1/5
100	5/5	4/5
200	4/5*(a)*	5/5*(a)*
400	1/5*(a)*	3/5*(a)*
Females		
25	0/5	0/5
50	5/5	0/5
100	5/5	2/5
200	3/5*(a)*	4/5*(a)*
400	3/5*(a)*	4/5*(a)*

(a) See Table 2 for days of death.

Thirteen-Week Study

No compound-related deaths or histopathologic effects in the stomach or other tissues were observed. Mean body weight gains of control and dosed groups were comparable (Table 4). In this study, the highest dose level (25 mg/kg) had no effect on either male or female F344/N rats.

Doses of 12 and 25 mg/kg allyl isothiocyanate, administered five times per week by gavage, were selected for rats in the chronic study because compound-related gross pathologic effects were observed in the 14-day study at 50 mg/kg.

TABLE 4. DOSAGE, SURVIVAL, AND MEAN BODY WEIGHTS OF RATS ADMINISTERED ALLYL ISOTHIOCYANATE BY GAVAGE FOR 13 WEEKS

Dose (mg/kg) *(a)*	Survival *(b)*	Mean Body Weight (grams)			Weight Change Relative to Controls *(d)* (Percent)
		Initial	Final	Change *(c)*	
Males					
0*(e)*	10/10	65.4 ± 3.4	309.8 ± 5.4	+244.4 ± 3.8	
1.5	10/10	65.9 ± 2.8	322.5 ± 6.2	+256.6 ± 4.8	+5.0
3	10/10	67.2 ± 2.6	321.0 ± 5.2	+253.8 ± 4.2	+3.8
6	10/10	67.2 ± 3.9	318.4 ± 5.4	+251.2 ± 4.9	+2.8
12	10/10	66.9 ± 2.9	314.5 ± 5.4	+247.6 ± 4.8	+1.3
25	10/10	66.7 ± 4.4	303.4 ± 8.8	+236.7 ± 7.5	-3.2
Females					
0*(e)*	10/10	56.1 ± 1.8	191.9 ± 3.1	+135.8 ± 4.1	
1.5	10/10	60.0 ± 2.1	194.7 ± 4.4	+134.7 ± 5.1	-0.8
3	10/10	64.0 ± 2.3	196.4 ± 4.0	+132.4 ± 4.1	-2.5
6	10/10	60.8 ± 2.4	195.3 ± 3.6	+134.5 ± 2.1	-1.0
12	10/10	59.8 ± 1.9	191.4 ± 3.0	+131.6 ± 3.8	-3.1
25	10/10	62.6 ± 2.7	192.9 ± 4.4	+130.3 ± 3.3	-4.1

(a) Allyl isothiocyanate in corn oil was administered 5 days per week.
(b) Number surviving/number initially in the group.
(c) Mean weight change of the group ± standard error of the mean.
(d) Weight change of the dosed group relative to that of the controls ■

$$\frac{\text{Weight Change (Dosed Group)} - \text{Weight Change (Control Group)}}{\text{Weight Change (Control Group)}} \times 100$$

(e) Vehicle controls received corn oil alone.

CHRONIC STUDY

Body Weights and Clinical Signs

Throughout the study, the mean body weights of high-dose male rats were lower than those of the controls, and during the last half of the study the mean body weights of both low- and high-dose female rats were higher than those of the controls (Figure 1, and Appendix H, Table H1). No compound-related clinical signs were observed.

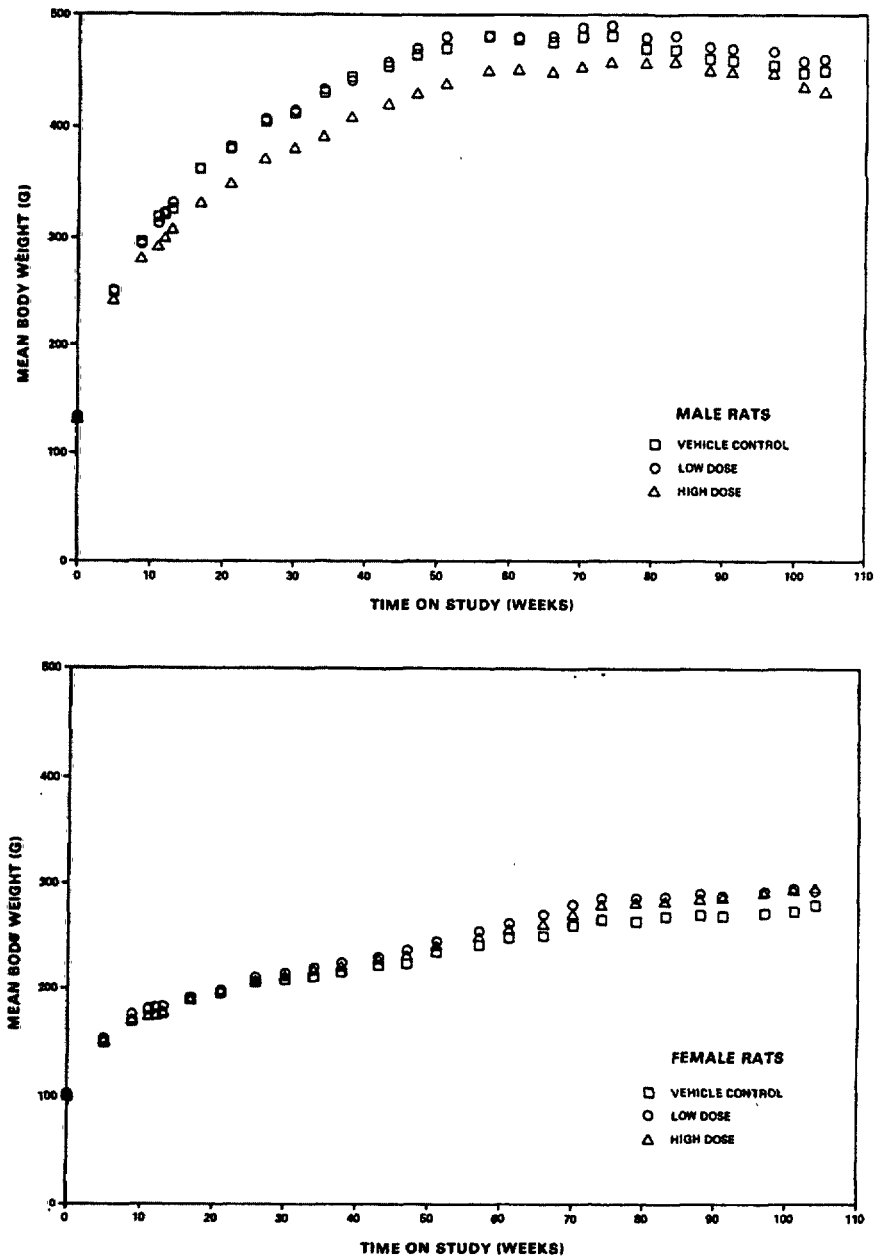


Figure 1. Growth Curves for Rats Administered Allyl Isothiocyanate by Gavage.

Survival

Estimates of the probabilities of survival of male and female rats administered allyl isothiocyanate by gavage at the doses of this bioassay, together with those of the control groups, are shown by the Kaplan and Meier curves in Figure 2. Two male rats were accidentally killed, one in the low-dose group at week 54 and one in the high-dose group at week 68. Two female rats in the low-dose group were accidentally killed at week 54. These deaths were due to gavage error. No significant differences in survival were observed. One control male, one low-dose male, and two low-dose females died during weeks 104-106. In the statistical analyses reported in Tables 6 and 7, no distinction was made between these animals and those killed during the termination period.

In male rats, 37/50 (74%) of the controls, 32/50 (64%) of the low-dose, and 33/50 (66%) of the high-dose group lived to the termination period of the study at 104-106 weeks. In female rats, 35/50 (70%) of the controls, 29/50 (58%) of the low-dose, and 33/50 (66%) of the high-dose group lived to the end of the study at 104-106 weeks.

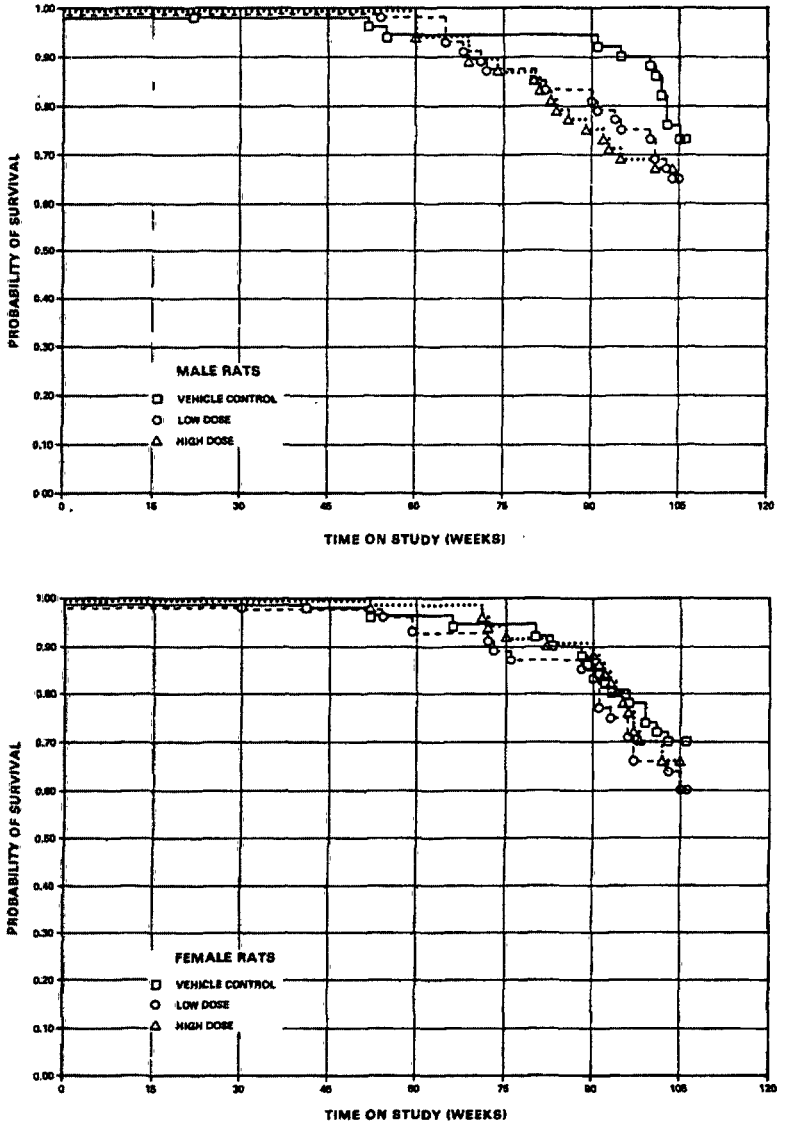


Figure 2. Survival Curves for Rats Administered Allyl Isothiocyanate by Gavage.

Pathology and Statistical Analyses of Results

Histopathologic findings on neoplasms in rats are summarized in Appendix A, Tables A1 and A2; Tables A3 and A4 give the survival and tumor status for each individual animal in the male rat and female rat studies, respectively. Findings on nonneoplastic lesions are summarized in Appendix C, Tables C1 and C2. Tables 6 and 7 contain the statistical analyses of those primary tumors that occurred with an incidence of at least 5% in one of the three groups.

Subcutaneous Tissue: Fibrosarcomas were observed in 3/50 (6%) high-dose female rats; none were seen in the control and low-dose groups. The results of all three trend tests were significant ($P<0.05$), but comparisons between the high-dose and control groups were not significant.

Hematopoietic System: Leukemia was observed in dosed male rats with a statistically significant positive trend ($P<0.05$; incidence: control, 2/50, 4%; low-dose 6/50, 12%; high-dose, 8/50, 16%). The incidence in the male high-dose group was significantly higher ($P<0.05$) than that in the control group. This leukemia, designated here as undifferentiated leukemia, is the typical leukemia of F344/N rats and is variously described as mononuclear cell leukemia. Fischer rat leukemia, or monocytic leukemia.

Urinary Bladder: Transitional-cell papillomas occurred in dosed male rats with a statistically significant ($P<0.05$) positive trend. Incidences in the control, low-dose, and high-dose groups were 0/49 (0%), 2/49 (4%), and 4/49 (8%). One female rat in the high-dose group had this lesion; the results in female rats were not significant. Epithelial hyperplasia was seen in 1/49 (2%) low-dose and 6/49 (12%) high-dose male rats. Both the overall trend and the increase at the high dose were statistically significant ($P<0.05$). Incidences of bladder lesions are given in Table 5.

Three of the tumors were large polypoid masses. The other lesions were small. Two of the large papillomas had a prominent myxomatous stroma. The hyperplasias were focal and small; a few were associated with mild inflammation. Urinary calculi were not observed in any animals in this study.

Eye: An increased incidence of nonneoplastic lesions consisting of retinopathy and cataract formation was observed in high-dose male rats and in low-dose female rats. Retinopathy was seen in 9/50 (18%) control males, 6/50 (12%) low-dose males, 39/50 (78%) high-dose males, 4/50 (8%) control females, 35/50 (70%) low-dose females, and 11/50 (22%) high-dose females. Cataract formation was observed in 7/50 (14%) control males, 6/50 (12%) low-dose males, 13/50 (26%) high-dose males, 2/50 (4%) control females, 33/50 (66%) low-dose females, and 9/50 (18%) high-dose females. The incidence of retinopathy and cataract formation correlated with the placement of the cages. The animals that occupied the two top levels of the racks (i.e., high-dose males and low-dose females) had the highest incidence of eye effects.

TABLE 5. INCIDENCE OF RATS WITH BLADDER LESIONS IN THE CHRONIC STUDY WITH ALLYL ISOTHIOCYANATE

	Incidence					
	Males			Females		
	Vehicle Control	Low Dose	High Dose	Vehicle Control	Low Dose	High Dose
Animals examined	49	49	49	49	49	50
Lesion:						
Transitional-Cell Papilloma	0	2	4	0	0	1
Epithelial Hyperplasia	0	1	6 *(a)*	0	0	1
Nodular Hyperplasia	0	0	1	0	0	0

(a) None of these animals had papillomas.

TABLE 6. ANALYSIS OF PRIMARY TUMORS IN MALE RATS *(a)*

	Vehicle Control	Low Dose	High Dose
Skin: Squamous Cell Papilloma			
Tumor Rates			
Overall *(b)*	3/50 (6%)	0/50 (0%)	4/50 (8%)
Adjusted *(c)*	7.6%	0.0%	12.1%
Terminal *(d)*	2/38 (5%)	0/33 (0%)	4/33 (12%)
Statistical Tests *(e)*			
Life Table	P=0.331	P=0.152N	P=0.418
Incidental Tumor Test	P=0.292	P=0.159N	P=0.364
Cochran-Armitage Trend, Fisher Exact Tests	P=0.393	P=0.121N	P=0.500
Skin: Squamous Cell Papilloma or Carcinoma			
Tumor Rates			
Overall *(b)*	4/50 (8%)	0/50 (0%)	6/50 (12%)
Adjusted *(c)*	10.1%	0.0%	17.2%
Terminal *(d)*	3/38 (8%)	0/33 (0%)	5/33 (15%)
Statistical Tests *(e)*			
Life Table	P=0.203N	P=0.086N	P=0.284
Incidental Tumor Test	P=0.234N	P=0.090N	P=0.331
Cochran-Armitage Trend, Fisher Exact Tests	P=0.260	P=0.059N	P=0.370
Subcutaneous Tissue: Fibrosarcoma			
Tumor Rates			
Overall *(b)*	5/50 (10%)	5/50 (10%)	1/50 (2%)
Adjusted *(c)*	12.5%	14.1%	2.8%
Terminal *(d)*	4/38 (11%)	4/33 (12%)	0/33 (0%)
Statistical Tests *(e)*			
Life Table	P=0.133N	P=0.542	P=0.154N
Incidental Tumor Test	P=0.123N	P=0.628N	P=0.215N
Cochran-Armitage Trend, Fisher Exact Tests	P=0.088N	P=0.630	P=0.102N
Subcutaneous Tissue: All Sarcomas			
Tumor Rates			
Overall *(b)*	6/50 (12%)	8/50 (16%)	2/50 (4%)
Adjusted *(c)*	14.5%	20.5%	5.1%
Terminal *(d)*	4/38 (11%)	5/33 (15%)	0/33 (0%)
Statistical Tests *(e)*			
Life Table	P=0.189N	P=0.304	P=0.209N
Incidental Tumor Test	P=0.088N	P=0.540	P=0.198N
Cochran-Armitage Trend, Fisher Exact Tests	P=0.124N	P=0.387	P=0.134N
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma			
Tumor Rates			
Overall *(b)*	3/49 (6%)	2/49 (4%)	3/48 (6%)
Adjusted *(c)*	7.2%	6.3%	8.8%
Terminal *(d)*	1/37 (3%)	2/32 (6%)	2/31 (6%)
Statistical Tests *(e)*			
Life Table	P=0.512	P=0.556N	P=0.590
Incidental Tumor Test	P=0.545N	P=0.426N	P=0.541N
Cochran-Armitage Trend, Fisher Exact Tests	P=0.577	P=0.500N	P=0.651

TABLE 6. ANALYSIS OF PRIMARY TUMORS IN MALE RATS *(a)* (Continued)

	Vehicle Control	Low Dose	High Dose
Hematopoietic System: Undifferentiated Leukemia			
Tumor Rates			
Overall *(b)*	2/50 (4%)	6/50 (12%)	8/50 (16%)
Adjusted *(c)*	4.7%	17.1%	21.6%
Terminal *(d)*	0/38 (0%)	4/33 (12%)	5/33 (15%)
Statistical Tests *(e)*			
Life Table	P=0.024	P=0.093	P=0.030
Incidental Tumor Test	P=0.006	P=0.070	P=0.009
Cochran-Armitage Trend, Fisher Exact Tests	P=0.039	P=0.134	P=0.046
Hematopoietic System: Lymphoma or Leukemia			
Tumor Rates			
Overall *(b)*	2/50 (4%)	7/50 (14%)	8/50 (16%)
Adjusted *(c)*	4.7%	19.1%	21.6%
Terminal *(d)*	0/38 (0%)	4/33 (12%)	5/33 (15%)
Statistical Tests *(e)*			
Life Table	P=0.027	P=0.054	P=0.030
Incidental Tumor Test	P=0.011	P=0.060	P=0.009
Cochran-Armitage Trend, Fisher Exact Tests	P=0.044	P=0.080	P=0.046
Liver: Neoplastic Nodule			
Tumor Rates			
Overall *(b)*	2/50 (4%)	0/50 (0%)	5/50 (10%)
Adjusted *(c)*	5.3%	0.0%	15.2%
Terminal *(d)*	2/38 (5%)	0/33 (0%)	5/33 (15%)
Statistical Tests *(e)*			
Life Table	P=0.085	P=0.270N	P=0.162
Incidental Tumor Test	P=0.085	P=0.270N	P=0.162
Cochran-Armitage Trend, Fisher Exact Tests	P=0.112	P=0.247N	P=0.218
Urinary Bladder: Transitional-Cell Papilloma			
Tumor Rates			
Overall *(b)*	0/49 (0%)	2/49 (4%)	4/49 (8%)
Adjusted *(c)*	0.0%	5.5%	12.1%
Terminal *(d)*	0/37 (0%)	1/32 (3%)	4/33 (12%)
Statistical Tests *(e)*			
Life Table	P=0.030	P=0.209	P=0.049
Incidental Tumor Test	P=0.048	P=0.356	P=0.049
Cochran-Armitage Trend, Fisher Exact Tests	P=0.038	P=0.247	P=0.059
Pituitary: Adenoma			
Tumor Rates			
Overall *(b)*	7/47 (15%)	12/49 (24%)	4/49 (8%)
Adjusted *(c)*	18.0%	30.6%	11.7%
Terminal *(d)*	5/36 (14%)	6/32 (19%)	3/33 (9%)
Statistical Tests *(e)*			
Life Table	P=0.326N	P=0.107	P=0.336N
Incidental Tumor Test	P=0.270N	P=0.236	P=0.462N
Cochran-Armitage Trend, Fisher Exact Tests	P=0.204N	P=0.178	P=0.238N

TABLE 6. ANALYSIS OF PRIMARY TUMORS IN MALE RATS *(a)* (Continued)

	Vehicle Control	Low Dose	High Dose
Pituitary: Adenoma or Carcinoma			
Tumor Rates			
Overall *(b)*	7/47 (15%)	13/49 (27%)	4/49 (8%)
Adjusted *(c)*	18.0%	33.3%	11.7%
Terminal *(d)*	5/36 (14%)	7/32 (22%)	3/33 (9%)
Statistical Tests *(e)*			
Life Table	P=0.329N	P=0.071	P=0.336N
Incidental Tumor Test	P=0.275N	P=0.162	P=0.462N
Cochran-Armitage Trend, Fisher Exact Tests	P=0.205N	P=0.124	P=0.238N
Adrenal: Pheochromocytoma			
Tumor Rates			
Overall *(b)*	16/50 (32%)	15/50 (30%)	11/50 (22%)
Adjusted *(c)*	39.7%	40.8%	33.3%
Terminal *(d)*	14/38 (37%)	12/33 (36%)	11/33 (33%)
Statistical Tests *(e)*			
Life Table	P=0.293N	P=0.483	P=0.322N
Incidental Tumor Test	P=0.260N	P=0.580N	P=0.376N
Cochran-Armitage Trend, Fisher Exact Tests	P=0.158N	P=0.500N	P=0.184N
Adrenal: Pheochromocytoma or Malignant Pheochromocytoma			
Tumor Rates			
Overall *(b)*	17/50 (34%)	15/50 (30%)	11/50 (22%)
Adjusted *(c)*	41.1%	40.8%	33.3%
Terminal *(d)*	14/38 (37%)	12/33 (36%)	11/33 (33%)
Statistical Tests *(e)*			
Life Table	P=0.231N	P=0.557	P=0.258N
Incidental Tumor Test	P=0.213N	P=0.505N	P=0.330N
Cochran-Armitage Trend, Fisher Exact Tests	P=0.113N	P=0.415N	P=0.133N
Thyroid: C-Cell Adenoma			
Tumor Rates			
Overall *(b)*	6/48 (13%)	10/50 (20%)	5/50 (10%)
Adjusted *(c)*	16.7%	29.1%	14.6%
Terminal *(d)*	6/36 (17%)	9/33 (27%)	4/33 (12%)
Statistical Tests *(e)*			
Life Table	P=0.511N	P=0.151	P=0.570N
Incidental Tumor Test	P=0.470N	P=0.194	P=0.614N
Cochran-Armitage Trend, Fisher Exact Tests	P=0.400N	P=0.233	P=0.471N
Thyroid: C-Cell Adenoma or Carcinoma			
Tumor Rates			
Overall *(b)*	8/48 (17%)	11/50 (22%)	7/50 (14%)
Adjusted *(c)*	21.4%	30.7%	20.5%
Terminal *(d)*	7/36 (19%)	9/33 (27%)	6/33 (18%)
Statistical Tests *(e)*			
Life Table	P=0.530N	P=0.235	P=0.587N
Incidental Tumor Test	P=0.474N	P=0.348	P=0.560
Cochran-Armitage Trend, Fisher Exact Tests	P=0.404N	P=0.341	P=0.465N

TABLE 6. ANALYSIS OF PRIMARY TUMORS IN MALE RATS *(a)* (Continued)

	Vehicle Control	Low Dose	High Dose
Pancreatic Islets: Islet-Cell Adenoma or Carcinoma			
Tumor Rates			
Overall *(b)*	3/50 (6%)	2/50 (4%)	1/49 (2%)
Adjusted *(c)*	7.9%	6.1%	3.0%
Terminal *(d)*	3/38 (8%)	2/33 (6%)	1/33 (3%)
Statistical Tests *(e)*			
Life Table	P=0.272N	P=0.564N	P=0.356N
Incidental Tumor Test	P=0.272N	P=0.564N	P=0.356N
Cochran-Armitage Trend, Fisher Exact Tests	P=0.232N	P=0.500N	P=0.316N
Mammary Gland: Fibroadenoma			
Tumor Rates			
Overall *(b)*	3/50 (6%)	3/50 (6%)	3/50 (6%)
Adjusted *(c)*	7.4%	9.1%	9.1%
Terminal *(d)*	2/38 (5%)	3/33 (9%)	3/33 (9%)
Statistical Tests *(e)*			
Life Table	P=0.508	P=0.591	P=0.584
Incidental Tumor Test	P=0.474	P=0.584	P=0.533
Cochran-Armitage Trend, Fisher Exact Tests	P=0.586	P=0.661	P=0.661
Preputial Gland: Adenocarcinoma			
Tumor Rates			
Overall *(b)*	4/50 (8%)	1/50 (2%)	1/50 (2%)
Adjusted *(c)*	10.5%	3.0%	3.0%
Terminal *(d)*	4/38 (11%)	1/33 (3%)	1/33 (3%)
Statistical Tests *(e)*			
Life Table	P=0.137N	P=0.223N	P=0.223N
Incidental Tumor Test	P=0.137N	P=0.223N	P=0.223N
Cochran-Armitage Trend, Fisher Exact Tests	P=0.108N	P=0.181N	P=0.181N
Preputial Gland: Carcinoma or Adenocarcinoma			
Tumor Rates			
Overall *(b)*	4/50 (8%)	2/50 (4%)	2/50 (4%)
Adjusted *(c)*	10.5%	6.1%	6.1%
Terminal *(d)*	4/38 (11%)	2/33 (6%)	2/33 (6%)
Statistical Tests *(e)*			
Life Table	P=0.316N	P=0.403N	P=0.403N
Incidental Tumor Test	P=0.316N	P=0.403N	P=0.403N
Cochran-Armitage Trend, Fisher Exact Tests	P=0.260N	P=0.339N	P=0.339N
Testis: Interstitial-Cell Tumor			
Tumor Rates			
Overall *(b)*	45/50 (90%)	45/50 (90%)	49/49 (100%)
Adjusted *(c)*	97.8%	95.7%	100.0%
Terminal *(d)*	37/38 (97%)	31/33 (94%)	33/33 (100%)
Statistical Tests *(e)*			
Life Table	P=0.024	P=0.146	P=0.023
Incidental Tumor Test	P=0.066	P=0.596N	P=0.068
Cochran-Armitage Trend, Fisher Exact Tests	P=0.036	P=0.630	P=0.030

TABLE 6. ANALYSIS OF PRIMARY TUMORS IN MALE RATS *(a)* (Continued)

(a) Dosed groups received doses of 12 or 25 mg/kg of allyl isothiocyanate by gavage.
(b) Number of tumor-bearing animals/number of animals examined at the site (percent).
(c) Kaplan-Meier estimated lifetime tumor incidence after adjusting for intercurrent mortality.
(d) Observed tumor incidence in surviving animals killed at end of study.
(e) Beneath the control incidence are the P-values associated with the trend test. Beneath the dosed group incidence are the P-values corresponding to pairwise comparisons between that dosed group and the control. The life table analysis regards tumors in animals dying before the end of the study as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Cochran-Armitage and Fisher's exact tests compare directly the overall incidence rates. A negative trend is indicated by (N).

TABLE 7. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS (a)

	Vehicle Control	Low Dose	High Dose
Subcutaneous Tissue: Fibrosarcoma			
Tumor Rates			
Overall *(b)*	0/50 (0%)	0/50 (0%)	3/50 (6%)
Adjusted *(c)*	0.0%	0.0%	8.1%
Terminal *(d)*	0/35 (0%)	0/31 (0%)	2/33 (6%)
Statistical Tests *(e)*			
Life Table	P=0.037	*(f)*	P=0.116
Incidental Tumor Test	P=0.028	*(f)*	P=0.094
Cochran-Armitage Trend, Fisher Exact Tests	P=0.036	*(f)*	P=0.121
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma			
Tumor Rates			
Overall *(b)*	1/50 (2%)	0/50 (0%)	3/50 (6%)
Adjusted *(c)*	2.9%	0.0%	7.4%
Terminal *(d)*	1/35 (3%)	0/31 (0%)	1/33 (3%)
Statistical Tests *(e)*			
Life Table	P=0.174	P=0.524N	P=0.301
Incidental Tumor Test	P=0.125	P=0.524N	P=0.223
Cochran-Armitage Trend, Fisher Exact Tests	P=0.171	P=0.500N	P=0.309
Hematopoietic System: Undifferentiated Leukemia			
Tumor Rates			
Overall *(b)*	7/50 (14%)	9/50 (18%)	11/50 (22%)
Adjusted *(c)*	16.6%	23.8%	26.1%
Terminal *(d)*	3/35 (9%)	4/31 (13%)	4/33 (12%)
Statistical Tests *(e)*			
Life Table	P=0.192	P=0.318	P=0.219
Incidental Tumor Test	P=0.186	P=0.373	P=0.291
Cochran-Armitage Trend, Fisher Exact Tests	P=0.184	P=0.393	P=0.218
Hematopoietic System: All Leukemia			
Tumor Rates			
Overall *(b)*	7/50 (14%)	9/50 (18%)	12/50 (24%)
Adjusted *(c)*	16.6%	23.8%	28.6%
Terminal *(d)*	3/35 (9%)	4/31 (13%)	5/33 (15%)
Statistical Tests *(e)*			
Life Table	P=0.136	P=0.318	P=0.159
Incidental Tumor Test	P=0.124	P=0.373	P=0.210
Cochran-Armitage Trend, Fisher Exact Tests	P=0.125	P=0.393	P=0.154
Hematopoietic System: Lymphoma or Leukemia			
Tumor Rates			
Overall *(b)*	8/50 (14%)	9/50 (18%)	14/50 (28%)
Adjusted *(c)*	19.2%	23.8%	31.6%
Terminal *(d)*	4/35 (11%)	4/31 (13%)	5/33 (15%)
Statistical Tests *(e)*			
Life Table	P=0.101	P=0.410	P=0.125
Incidental Tumor Test	P=0.096	P=0.479	P=0.206
Cochran-Armitage Trend, Fisher Exact Tests	P=0.087	P=0.500	P=0.114

TABLE 7. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS *(a)* (Continued)

Topography:Morphology	Vehicle Control	Low Dose	High Dose
Pituitary: Adenoma			
Tumor Rates			
Overall *(b)*	17/49(35%)	10/50(20%)	13/50(26%)
Adjusted *(c)*	44.3%	29.8%	36.7%
Terminal *(d)*	13/34(38%)	8/31(26%)	11/33(33%)
Statistical Tests *(e)*			
Life Table	P=0.247N	P=0.145N	P=0.283N
Incidental Tumor Test	P=0.241N	P=0.139N	P=0.279N
Cochran-Armitage Trend, Fisher Exact Tests	P=0.207N	P=0.078N	P=0.235N
Pituitary: Carcinoma			
Tumor Rates			
Overall *(b)*	0/49(0%)	3/50(6%)	2/50(4%)
Adjusted *(c)*	0.0%	9.7%	6.1%
Terminal *(d)*	0/34(0%)	3/31(10%)	2/33(6%)
Statistical Tests *(e)*			
Life Table	P=0.208	P=0.105	P=0.231
Incidental Tumor Test	P=0.208	P=0.105	P=0.231
Cochran-Armitage Trend, Fisher Exact Tests	P=0.219	P=0.125	P=0.253
Pituitary: Adenoma or Carcinoma			
Tumor Rates			
Overall *(b)*	17/49(35%)	13/50(26%)	15/50(30%)
Adjusted *(c)*	44.3%	38.9%	42.5%
Terminal *(d)*	13/34(38%)	11/31(35%)	13/33(39%)
Statistical Tests *(e)*			
Life Table	P=0.407N	P=0.360N	P=0.446N
Incidental Tumor Test	P=0.404N	P=0.359N	P=0.447N
Cochran-Armitage Trend, Fisher Exact Tests	P=0.355N	P=0.235N	P=0.388N
Adrenal: Pheochromocytoma			
Tumor Rates			
Overall *(b)*	1/50(2%)	2/50(4%)	3/50(6%)
Adjusted *(c)*	2.3%	6.5%	9.1%
Terminal *(d)*	0/35(0%)	2/31(6%)	3/33(9%)
Statistical Tests *(e)*			
Life Table	P=0.216	P=0.464	P=0.293
Incidental Tumor Test	P=0.194	P=0.451	P=0.256
Cochran-Armitage Trend, Fisher Exact Tests	P=0.226	P=0.500	P=0.309
Adrenal: Pheochromocytoma and Malignant Pheochromocytoma			
Tumor Rates			
Overall *(b)*	2/50(4%)	2/50(4%)	3/50(6%)
Adjusted *(c)*	5.1%	6.5%	9.1%
Terminal *(d)*	1/35(3%)	2/31(6%)	3/33(9%)
Statistical Tests *(e)*			
Life Table	P=0.390	P=0.654	P=0.481
Incidental Tumor Test	P=0.364	P=0.644	P=0.442
Cochran-Armitage Trend, Fisher Exact Tests	P=0.408	P=0.691	P=0.500

TABLE 7. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS *(a)* (Continued)

	Vehicle Control	Low Dose	High Dose
Thyroid: C-Cell Adenoma			
Tumor Rates			
Overall *(b)*	10/50 (20%)	8/48 (17%)	6/50 (12%)
Adjusted *(c)*	28.6%	26.1%	18.2%
Terminal *(d)*	10/35 (29%)	7/29 (24%)	6/33 (18%)
Statistical Tests *(e)*			
Life Table	P=0.200N	P=0.570N	P=0.236N
Incidental Tumor Test	P=0.211N	P=0.574N	P=0.236N
Cochran-Armitage Trend, Fisher Exact Tests	P=0.173N	P=0.435N	P=0.207N
Thyroid: C-Cell Carcinoma			
Tumor Rates			
Overall *(b)*	2/50 (4%)	2/48 (4%)	3/50 (6%)
Adjusted *(c)*	5.7%	6.9%	9.1%
Terminal *(d)*	2/35 (6%)	2/29 (7%)	3/33 (9%)
Statistical Tests *(e)*			
Life Table	P=0.385	P=0.626	P=0.473
Incidental Tumor Test	P=0.385	P=0.626	P=0.473
Cochran-Armitage Trend, Fisher Exact Tests	P=0.409	P=0.676	P=0.500
Thyroid: C-Cell Adenoma or Carcinoma			
Tumor Rates			
Overall *(b)*	12/50 (24%)	10/48 (21%)	9/50 (18%)
Adjusted *(c)*	34.3%	32.8%	27.3%
Terminal *(d)*	12/35 (34%)	9/29 (31%)	9/33 (27%)
Statistical Tests *(e)*			
Life Table	P=0.314N	P=0.598	P=0.359N
Incidental Tumor Test	P=0.327N	P=0.595	P=0.359N
Cochran-Armitage Trend, Fisher Exact Tests	P=0.272N	P=0.447N	P=0.312N
Mammary Gland: Fibroadenoma			
Tumor Rates			
Overall *(b)*	8/50 (16%)	14/50 (28%)	11/50 (22%)
Adjusted *(c)*	21.8%	39.7%	30.7%
Terminal *(d)*	7/35 (20%)	11/31 (35%)	9/33 (27%)
Statistical Tests *(e)*			
Life Table	P=0.247	P=0.068	P=0.264
Incidental Tumor Test	P=0.246	P=0.115	P=0.246
Cochran-Armitage Trend, Fisher Exact Tests	P=0.285	P=0.114	P=0.306
Uterus: Endometrial Stromal Polyp			
Tumor Rates			
Overall *(b)*	14/50 (28%)	15/49 (31%)	16/50 (32%)
Adjusted *(c)*	38.9%	44.8%	42.4%
Terminal *(d)*	13/35 (37%)	13/31 (42%)	12/33 (36%)
Statistical Tests *(e)*			
Life Table	P=0.311	P=0.346	P=0.347
Incidental Tumor Test	P=0.374	P=0.420	P=0.400
Cochran-Armitage Trend, Fisher Exact Tests	P=0.375	P=0.474	P=0.414

TABLE 7. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS *(a)* (Continued)

(a) Dosed groups received doses of 12 or 25 mg/kg of allyl isothiocyanate by gavage.
(b) Number of tumor-bearing animals/number of animals examined at the site (percent).
(c) Kaplan-Meier estimated lifetime tumor incidence after adjusting for intercurrent mortality.
(d) Observed tumor incidence in surviving animals killed at end of study.
(e) Beneath the control incidence are the P-values associated with the trend test. Beneath the dosed group incidence are the P-values corresponding to pairwise comparisons between that dosed group and the control. The life table analysis regards tumors in animals dying before the end of the study as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Cochran-Armitage and Fisher's exact tests compare directly the overall incidence rates. A negative trend is indicated by (N).
(f) No test was performed because there was no incidence in the low-dose or vehicle control group.

PRECHRONIC STUDIES

Single-Dose Study

Two of five males receiving 400 mg/kg and 4/5 males and 5/5 females receiving 800 mg/kg died (Table 8). The following average weight increases over the initial weight (on day 0) were calculated at the end of the 16th day for the surviving male and female mice:

Dose (mg/kg)	Weight Increase (Percent)	
	Males	Females
50	2	18
100	17	22
200	24	13
400	21	11
800	38	—

Male and female mice exhibited a transient, dose-related toxicity which was most marked in the 100, 200, 400, and 800 mg/kg groups. This included inactivity, drooping eyelids, and ruffled fur.

The peritoneal cavities were examined in male mice administered 200, 400, or 800 mg/kg and in female mice administered 100, 200, or 400 mg/kg. The lower third of the mucosal surface of the stomach was thickened and necrotic. The stomach adhered to the peritoneal wall in male mice administered 400 or 800 mg/kg and in female mice administered 200 or 400 mg/kg. The severity of these effects was dose related.

The highest dosage levels producing no deaths were 200 mg/kg in the males and 400 mg/kg in the females. In addition, the 100, 200, 400, and 800 mg/kg levels produced toxicity. For these reasons, the highest dose level in the 14-day study was set at 50 mg/kg.

Fourteen-Day Study

One male mouse administered 50 mg/kg died (Table 9). A thickened area of mucosa in the nonglandular region of the stomach was observed in 4/5 males and 5/5 females administered 50 mg/kg. A thickened urinary bladder wall was seen in 4/5 males and 1/5 females administered 50 mg/kg. The average weight gain in the experimental groups varied from 3% to 16%.

No other signs of toxicity were observed. Due to the stomach and bladder lesions observed at the 50 mg/kg dose, the highest dose set for the 13-week study was 25 mg/kg.

Thirteen-Week Study

No compound-related deaths or histopathologic effects in the stomach or other tissues were observed. Mean body weight gains of control and dosed groups were comparable (Table 10). The highest dose level (25 mg/kg) had no effect on male or female B6C3F1 mice.

Doses of 12 and 25 mg/kg allyl isothiocyanate, administered five times per week by gavage, were selected for mice in the chronic study because compound-related effects were observed in the 14-day study at 50 mg/kg.

TABLE 8. DOSAGE AND SURVIVAL OF MICE ADMINISTERED A SINGLE DOSE OF ALLYL ISOTHIOCYANATE IN CORN OIL BY GAVAGE

Dose (mg/kg)	Survival *(a)*	
	Males	Females
50	5/5	5/5
100	5/5	5/5
200	5/5	5/5
400	3/5 *(b)*	5/5
800	1/5 *(c)*	0/5 *(d)*

(a) Number surviving/number initially in the group.
(b) Deaths occurred on days 1 and 14.
(c) Two animals died on day 1 and two animals on day 2.
(d) Four animals died on day 1 and one animal on day 2.

TABLE 9. DOSAGE, SURVIVAL, AND MEAN BODY WEIGHTS OF MICE RECEIVING ALLYL ISOTHIOCYANATE BY GAVAGE FOR 14 DAYS

Dose (mg/kg)	Survival (a)	Mean Body Weight (grams)		
		Initial	Final	Change (b)
Males				
3	5/5	20.2 ± 0.4	21.0 ± 0.7	+0.8 ± 0.5
6	5/5	20.6 ± 0.2	22.6 ± 0.7	+2.0 ± 0.5
12	5/5	20.2 ± 0.7	21.0 ± 1.0	+0.8 ± 0.4
25	5/5	19.8 ± 0.5	21.8 ± 0.7	+2.0 ± 0.5
50	4/5 (c)	20.5 ± 0.7	23.8 ± 0.5	+3.3 ± 0.8
Females				
3	5/5	17.4 ± 0.4	19.0 ± 0.3	+1.6 ± 0.5
6	5/5	16.6 ± 0.2	18.8 ± 0.7	+2.2 ± 0.7
12	5/5	17.8 ± 0.5	18.4 ± 0.4	+0.6 ± 0.2
25	5/5	16.8 ± 0.4	18.4 ± 0.2	+1.6 ± 0.5
50	5/5	17.6 ± 0.5	18.0 ± 0.9	+0.4 ± 1.0

(a) Number surviving/number initially in the group. All calculations refer only to the survivors of each group.
(b) Mean weight change of the survivors of the group ± standard error of the mean.
(c) Death occurred on day 15, the day after administration of the test material was discontinued.

TABLE 10. DOSAGE, SURVIVAL, AND MEAN BODY WEIGHTS OF MICE ADMINISTERED ALLYL ISOTHIOCYANATE BY GAVAGE FOR 13 WEEKS

Dose (a) (mg/kg)	Survival (b)	Mean Body Weight (grams)			Weight Change Relative to Controls (d) (Percent)
		Initial	Final	Change (c)	
Males					
0(e)	10/10	18.7 ±0.5	32.4 ±0.6	+13.7 ±0.5	
1.5	9/10 (f)	19.4 ±0.3	34.1 ±1.1	+14.7 ±1.1	+ 7.3
3	10/10	18.2 ±0.6	33.4 ±1.1	+15.2 ±0.8	+10.9
6	10/10	18.7 ±0.7	35.0 ±0.8	+16.3 ±0.8	+19.0
12	9/10 (f)	20.1 ±0.5	32.8 ±0.4	+12.7 ±0.4	- 7.3
25	10/10	19.9 ±0.4	35.2 ±0.6	+15.3 ±0.8	+11.7
Females					
0(e)	10/10	16.1 ±0.4	25.3 ±0.3	+9.2 ±0.4	
1.5	10/10	15.6 ±0.3	24.3 ±0.5	+8.7 ±0.7	- 5.4
3	8/10 (f)	16.4 ±0.5	24.5 ±0.6	+8.1 ±0.2	-12.0
6	9/10 (f)	16.6 ±0.4	25.2 ±0.6	+8.6 ±0.5	- 6.5
12	9/10 (f)	16.9 ±0.5	25.9 ±0.8	+9.0 ±0.7	- 2.2
25	10/10	15.9 ±0.4	24.5 ±0.5	+8.6 ±0.3	- 6.5

(a) Allyl isothiocyanate in corn oil was administered 5 days per week.
(b) Number surviving/number initially in the group. All calculations refer only to the survivors of each group.
(c) Mean weight change of the survivors of the group ± standard error of the mean.
(d) Weight change of the dosed group relative to that of the controls =

$$\frac{\text{Weight Change (Dosed Group)} - \text{Weight Change (Control Group)}}{\text{Weight Change (Control Group)}} \times 100$$

(e) Vehicle controls received corn oil alone.
(f) Death was a result of gavage error.

CHRONIC STUDY

Body Weights and Clinical Signs

Throughout most of the study, mean body weights of high-dose male and female mice were higher than those of the vehicle controls (Figure 3, Appendix H, Table H2).

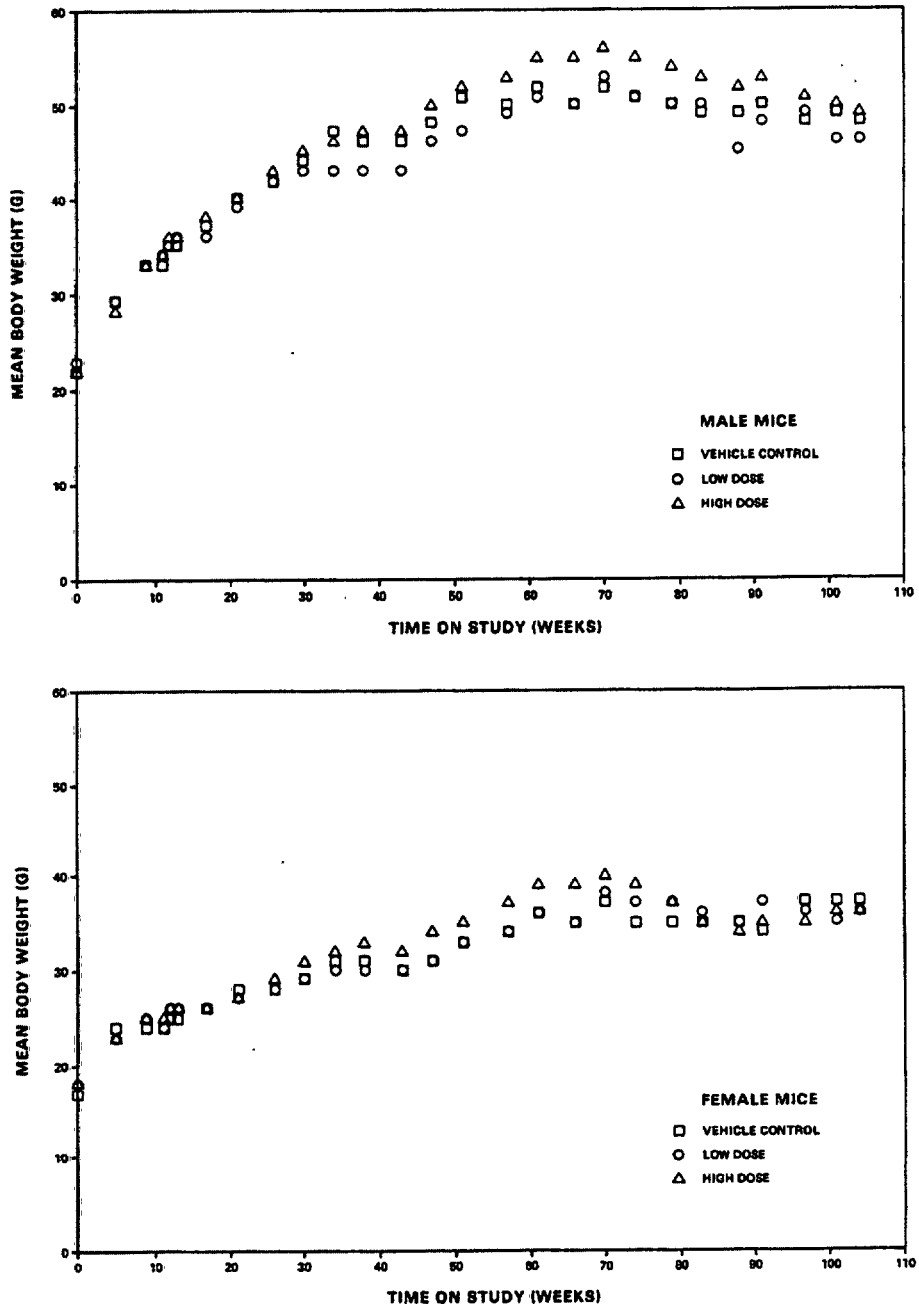
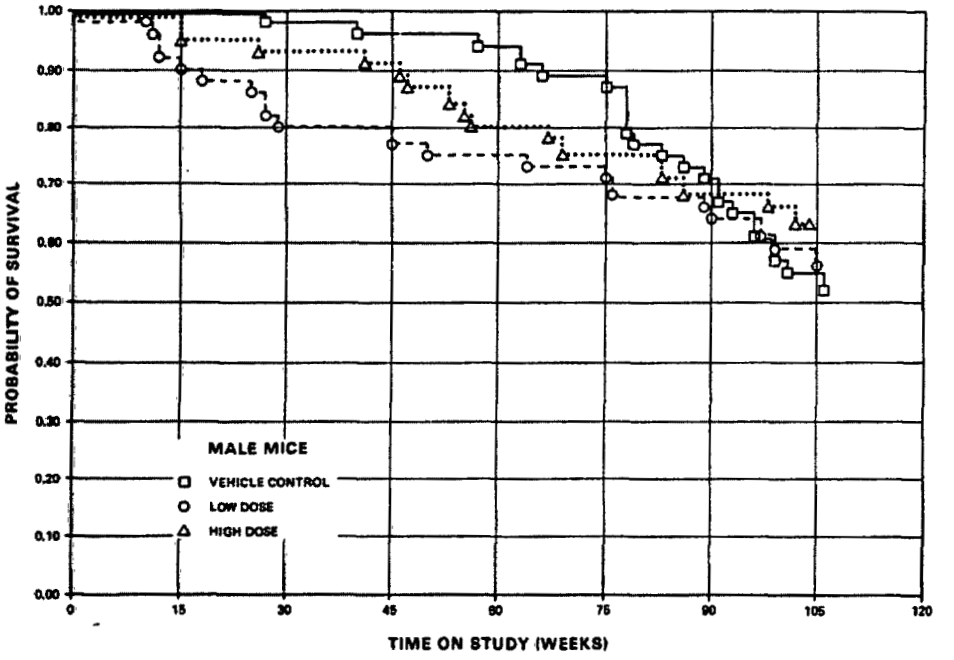


Figure 3. Growth Curves for Mice Administered Allyl Isothiocyanate by Gavage.

Survival

Estimates of the probabilities of survival of male and female mice administered allyl isothiocyanate by gavage at the doses of this bioassay, together with those of the control groups, are shown by the Kaplan and Meier curves in Figure 4. No significant differences in survival were observed between any groups of either sex. The survival in control female mice was consistently lower than the survival in either dosed group after week 40. One control male, one low-dose



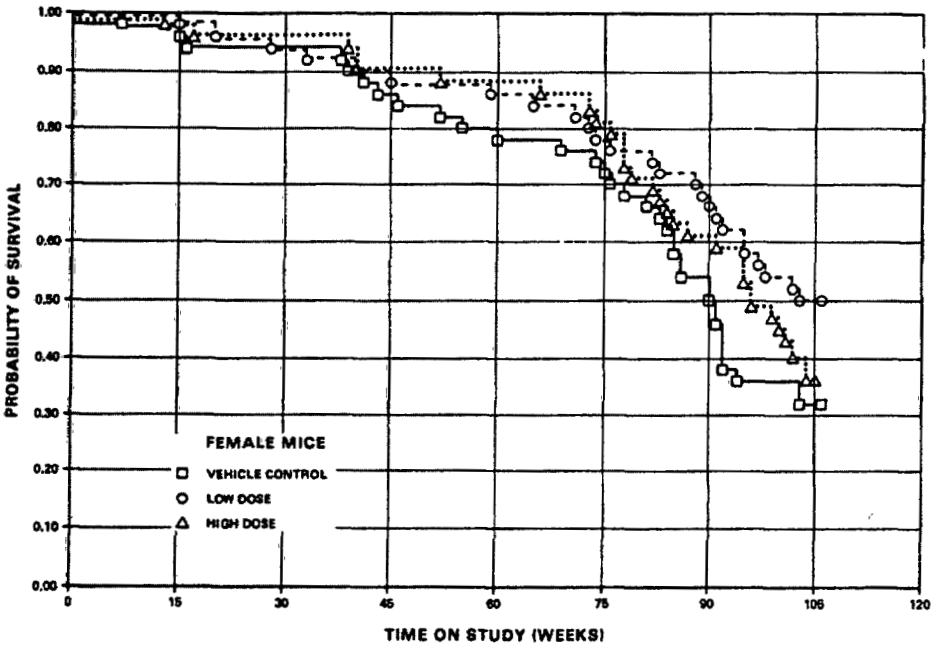


Figure 4. Survival Curves for Mice Administered Allyl Isothiocyanate by Gavage.

male, and two high-dose female mice died during weeks 104-106. In the statistical analyses reported in Tables 11 and 12, no distinction was made between these animals and those killed during this termination period. One control male (at week 41), six low-dose males (at weeks 42, 48, 56, 59, 60, and 65), seven high-dose males (at weeks 6, 20, 29, 31, 35, 62, and 65), and one high-dose female (at week 60) were accidentally killed (due to gavage error) during the study.

In male mice, 26/50 (52%) of the controls, 24/50 (48%) of the low-dose, and 27/50 (54%) of the high-dose group lived to the termination period of the study at 104-106 weeks. In female mice, 16/50 (32%) of the controls, 25/50 (50%) of the low-dose, and 18/50 (36%) of the high-dose group lived to the termination period of the study at 104-106 weeks. Suppurative inflammation of the peritoneum, uterus, or multiple organs was seen in many of the female mice that died before 104 weeks (13/34 controls, 6/25 low-dose, 12/30 high-dose). These lesions are suggestive of generalized infection and may have been causative in these early deaths.

Pathology and Statistical Analyses of Results

Histopathologic findings on neoplasms occurring in mice are summarized in Appendix B, Tables B1 and B2; Tables B3 and B4 give the survival and tumor status for each individual animal in the male and female mouse studies, respectively. Findings on nonneoplastic lesions are summarized in Appendix D, Tables D1 and D2. Tables 11 and 12 contain the statistical analyses of those primary tumors that occurred with an incidence of at least 5% in one of the three groups.

Liver: A significant, ($P<0.01$) dose-related increase in cytoplasmic vacuolization was observed in male mice (control 2/49, 4%; low-dose, 8/49, 16%; high-dose, 13/50, 26%). The distribution of these vacuoles was not consistent, but most livers had some centrilobular component. In other male mice with cytoplasmic vacuolization, the distribution was more consistently centrilobular. The vacuoles contained fat as determined by special stains of frozen sections. The degree of severity was similiar in the three groups.

TABLE 11. ANALYSIS OF PRIMARY TUMORS IN MALE MICE *(a)*

	Vehicle Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma without Carcinoma			
Tumor Rates			
Overall *(b)*	4/50 (8%)	3/50 (6%)	4/50 (8%)
Adjusted *(c)*	14.8%	10.6%	14.3%
Terminal *(d)*	4/27 (15%)	2/25 (8%)	3/27 (11%)
Statistical Tests *(e)*			
Life Table	P=0.435	P=0.557N	P=0.643N
Incidental Tumor Test	P=0.509	P=0.547N	P=0.575
Cochran-Armitage Trend, Fisher Exact Tests	P=0.575	P=0.500N	P=0.643
Lung: Alveolar/Bronchiolar Carcinoma			
Tumor Rates			
Overall *(b)*	0/50 (0%)	1/50 (2%)	3/50 (6%)
Adjusted *(c)*	0.0%	4.0%	10.3%
Terminal *(d)*	0/27 (0%)	1/25 (4%)	2/27 (7%)
Statistical Tests *(e)*			
Life Table	P=0.060	P=0.485	P=0.113
Incidental Tumor Test	P=0.048	P=0.485	P=0.084
Cochran-Armitage Trend, Fisher Exact Tests	P=0.061	P=0.500	P=0.121
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma			
Tumor Rates			
Overall *(b)*	4/50 (8%)	4/50 (8%)	7/50 (14%)
Adjusted *(c)*	14.8%	14.5%	23.9%
Terminal *(d)*	4/27 (15%)	3/25 (12%)	5/27 (19%)
Statistical Tests *(e)*			
Life Table	P=0.191	P=0.588	P=0.253
Incidental Tumor Test	P=0.143	P=0.598	P=0.176
Cochran-Armitage Trend, Fisher Exact Tests	P=0.201	P=0.643	P=0.262
Hematopoietic System: Lymphoma			
Tumor Rates			
Overall *(b)*	3/50 (6%)	2/50 (4%)	0/50 (0%)
Adjusted *(c)*	8.9%	7.7%	0.0%
Terminal *(d)*	1/27 (4%)	1/25 (4%)	0/27 (0%)
Statistical Tests *(e)*			
Life Table	P=0.104N	P=0.576N	P=0.148N
Incidental Tumor Test	P=0.175N	P=0.661	P=0.194N
Cochran-Armitage Trend, Fisher Exact Tests	P=0.083N	P=0.500N	P=0.121N
Liver: Adenoma without Carcinoma			
Tumor Rates			
Overall *(b)*	8/49 (16%)	5/49 (10%)	9/50 (18%)
Adjusted *(c)*	28.0%	18.7%	31.3%
Terminal *(d)*	7/27 (26%)	4/25 (16%)	8/27 (30%)
Statistical Tests *(e)*			
Life Table	P=0.411	P=0.349N	P=0.482
Incidental Tumor Test	P=0.439	P=0.378N	P=0.540
Cochran-Armitage Trend, Fisher Exact Tests	P=0.453	P=0.276N	P=0.518

TABLE 11. ANALYSIS OF PRIMARY TUMORS IN MALE MICE *(a)* (Continued)

	Vehicle Control	Low Dose	High Dose
Liver: Carcinoma			
Tumor Rates			
Overall *(b)*	13/49 (27%)	9/49 (18%)	10/50 (20%)
Adjusted *(c)*	35.3%	29.4%	35.7%
Terminal *(d)*	5/27 (19%)	5/25 (20%)	9/27 (33%)
Statistical Tests *(e)*			
Life Table	P=0.356N	P=0.408N	P=0.385N
Incidental Tumor Test	P=0.534N	P=0.580N	P=0.597
Cochran-Armitage Trend, Fisher Exact Tests	P=0.261N	P=0.234N	P=0.298N
Liver: Adenoma or Carcinoma			
Tumor Rates			
Overall *(b)*	21/49 (43%)	14/49 (29%)	19/50 (38%)
Adjusted *(c)*	57.2%	45.4%	65.2%
Terminal *(d)*	12/27 (44%)	9/25 (36%)	17/27 (63%)
Statistical Tests *(e)*			
Life Table	P=0.476N	P=0.259N	P=0.490N
Incidental Tumor Test	P=0.469	P=0.392N	P=0.529
Cochran-Armitage Trend, Fisher Exact Tests	P=0.362N	P=0.103N	P=0.387N
Thyroid: Follicular-Cell Adenoma			
Tumor Rates			
Overall *(b)*	3/50 (6%)	2/45 (4%)	1/50 (2%)
Adjusted *(c)*	11.1%	7.2%	3.7%
Terminal *(d)*	3/27 (11%)	1/24 (4%)	1/27 (4%)
Statistical Tests *(e)*			
Life Table	P=0.242N	P=0.576N	P=0.303N
Incidental Tumor Test	P=0.236N	P=0.569N	P=0.303N
Cochran-Armitage Trend, Fisher Exact Tests	P=0.228N	P=0.550N	P=0.309N
Harderian Gland: Adenoma or Cystadenoma			
Tumor Rates			
Overall *(b)*	3/50 (6%)	1/50 (2%)	1/50 (2%)
Adjusted *(c)*	10.0%	4.0%	3.7%
Terminal *(d)*	2/27 (7%)	1/25 (4%)	1/27 (4%)
Statistical Tests *(e)*			
Life Table	P=0.224N	P=0.346N	P=0.325N
Incidental Tumor Test	P=0.258N	P=0.420N	P=0.366N
Cochran-Armitage Trend, Fisher Exact Tests	P=0.210N	P=0.309N	P=0.309N

(a) Dosed groups received doses of 12 or 25 mg/kg of allyl isothiocyanate by gavage.
(b) Number of tumor-bearing animals/number of animals examined at the site (percent).
(c) Kaplan-Meier estimated lifetime tumor incidence after adjusting for intercurrent mortality.
(d) Observed tumor incidence in surviving animals killed at end of study.
(e) Beneath the control incidence are the P-values associated with the trend test. Beneath the dosed group incidence are the P-values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors in animals dying before the end of the study as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Cochran-Armitage and Fisher's exact tests compare directly the overall incidence rates. A negative trend is indicated by (N).

TABLE 12. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE *(a)*

	Vehicle Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Carcinoma			
Tumor Rates			
Overall *(b)*	0/47 (0%)	2/49 (4%)	3/49 (6%)
Adjusted *(c)*	0.0%	7.1%	11.8%
Terminal *(d)*	0/16 (0%)	0/25 (0%)	1/20 (5%)
Statistical Tests *(e)*			
Life Table	P=0.119	P=0.337	P=0.194
Incidental Tumor Test	P=0.247	P=0.395	P=0.281
Cochran-Armitage Trend, Fisher Exact Tests	P=0.091	P=0.258	P=0.129
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma			
Tumor Rates			
Overall *(b)*	2/47 (4%)	2/49 (4%)	3/49 (6%)
Adjusted *(c)*	7.9%	7.1%	11.8%
Terminal *(d)*	0/16 (0%)	0/25 (0%)	1/20 (5%)
Statistical Tests *(e)*			
Life Table	P=0.510	P=0.559N	P=0.626
Incidental Tumor Test	P=0.594	P=0.697N	P=0.600
Cochran-Armitage Trend, Fisher Exact Tests	P=0.425	P=0.676N	P=0.520
Hematopoietic System: Malignant Lymphoma, Lymphocytic Type			
Tumor Rates			
Overall *(b)*	3/50 (6%)	2/50 (4%)	1/49 (2%)
Adjusted *(c)*	13.6%	5.8%	5.0%
Terminal *(d)*	1/16 (6%)	0/25 (0%)	1/20 (5%)
Statistical Tests *(e)*			
Life Table	P=0.166N	P=0.354N	P=0.241N
Incidental Tumor Test	P=0.277N	P=0.604N	P=0.397N
Cochran-Armitage Trend, Fisher Exact Tests	P=0.232N	P=0.500N	P=0.316N
Hematopoietic System: Lymphoma			
Tumor Rates			
Overall *(b)*	5/50 (10%)	4/50 (8%)	4/49 (8%)
Adjusted *(c)*	21.3%	11.7%	17.9%
Terminal *(d)*	1/16 (6%)	1/25 (4%)	3/20 (15%)
Statistical Tests *(e)*			
Life Table	P=0.326N	P=0.320N	P=0.375N
Incidental Tumor Test	P=0.393N	P=0.562N	P=0.448N
Cochran-Armitage Trend, Fisher Exact Tests	P=0.447N	P=0.500N	P=0.513N
Hematopoietic System: Lymphoma or Leukemia			
Tumor Rates			
Overall *(b)*	5/50 (10%)	4/50 (8%)	6/49 (12%)
Adjusted *(c)*	21.3%	11.7%	24.6%
Terminal *(d)*	1/16 (6%)	1/25 (4%)	3/20 (15%)
Statistical Tests *(e)*			
Life Table	P=0.559	P=0.320N	P=0.593N
Incidental Tumor Test	P=0.559	P=0.562N	P=0.589N
Cochran-Armitage Trend, Fisher Exact Tests	P=0.418	P=0.500N	P=0.486

TABLE 12. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE *(a)* (Continued)

	Vehicle Control	Low Dose	High Dose
Liver: Adenoma or Carcinoma			
Tumor Rates			
Overall *(b)*	2/50 (4%)	3/49 (6%)	1/49 (2%)
Adjusted *(c)*	12.5%	10.9%	2.9%
Terminal *(d)*	2/16 (13%)	2/25 (8%)	0/20 (0%)
Statistical Tests *(e)*			
Life Table	P=0.325N	P=0.675N	P=0.445N
Incidental Tumor Test	P=0.453N	P=0.597	P=0.534N
Cochran-Armitage Trend, Fisher Exact Tests	P=0.404N	P=0.490	P=0.508N
Pituitary: Adenoma			
Tumor Rates			
Overall *(b)*	3/47 (6%)	3/45 (7%)	4/44 (9%)
Adjusted *(c)*	18.8%	11.0%	17.9%
Terminal *(d)*	3/16 (19%)	2/25 (8%)	3/20 (15%)
Statistical Tests *(e)*			
Life Table	P=0.535	P=0.465N	P=0.643
Incidental Tumor Test	P=0.493	P=0.561N	P=0.635N
Cochran-Armitage Trend, Fisher Exact Tests	P=0.388	P=0.641	P=0.463
Pituitary: Carcinoma			
Tumor Rates			
Overall *(b)*	3/47 (6%)	3/45 (7%)	0/44 (0%)
Adjusted *(c)*	18.8%	12.0%	0.0%
Terminal *(d)*	3/16 (19%)	3/25 (12%)	0/20 (0%)
Statistical Tests *(e)*			
Life Table	P=0.054N	P=0.444N	P=0.081N
Incidental Tumor Test	P=0.054N	P=0.444N	P=0.081N
Cochran-Armitage Trend, Fisher Exact Tests	P=0.112N	P=0.641	P=0.133N
Pituitary: Adenoma or Carcinoma			
Tumor Rates			
Overall *(b)*	6/47 (13%)	6/45 (13%)	4/44 (9%)
Adjusted *(c)*	37.5%	22.6%	17.9%
Terminal *(d)*	6/16 (38%)	5/25 (20%)	3/20 (15%)
Statistical Tests *(e)*			
Life Table	P=0.176N	P=0.304N	P=0.212N
Incidental Tumor Test	P=0.200N	P=0.371N	P=0.183N
Cochran-Armitage Trend, Fisher Exact Tests	P=0.354N	P=0.589	P=0.413N
Thyroid: Follicular-Cell Adenoma or Carcinoma			
Tumor Rates			
Overall *(b)*	1/48 (2%)	3/47 (6%)	3/47 (6%)
Adjusted *(c)*	6.3%	12.5%	15.0%
Terminal *(d)*	1/16 (6%)	3/24 (12%)	3/20 (15%)
Statistical Tests *(e)*			
Life Table	P=0.302	P=0.458	P=0.385
Incidental Tumor Test	P=0.302	P=0.458	P=0.385
Cochran-Armitage Trend, Fisher Exact Tests	P=0.238	P=0.300	P=0.300

TABLE 12. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE *(a)* (Continued)

(a) Dosed groups received doses of 12 or 25 mg/kg of allyl isothiocyanate by gavage.
(b) Number of tumor-bearing animals/number of animals examined at the site (percent).
(c) Kaplan-Meier estimated lifetime tumor incidence after adjusting for intercurrent mortality.
(d) Observed tumor incidence in surviving animals killed at end of study.
(e) Beneath the control incidence are the P-values associated with the trend test. Beneath the dosed group incidence are the P-values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors in animals dying before the end of the study as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Cochran-Armitage and Fisher's exact tests compare directly the overall incidence rates. A negative trend is indicated by (N).

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IV. DISCUSSION AND CONCLUSIONS

IV. DISCUSSION AND CONCLUSIONS

A 2-year carcinogenesis bioassay of allyl isothiocyanate was conducted in F344/N rats and B6C3F1 mice. Doses of 12 or 25 mg/kg allyl isothiocyanate, administered 5 times per week by gavage, were selected for the chronic study since the 50 mg/kg dose administered in the 14-day study produced thickening of the mucosal surface of the stomach in male and female rats and mice, adherence of the stomach to the peritoneum in male rats, and a thickened urinary bladder wall in male mice. A dose of 25 mg/kg produced no gross lesions when administered for 14 consecutive days or when administered 5 times per week for 13 weeks, and all animals survived this dose.

Survival of dosed and control rats was comparable in the chronic study. Throughout the study, the mean body weights of high-dose male rats were lower than those of controls, and during the last half of the study the mean body weights of high-dose female rats were higher than the control values.

Transitional-cell papillomas of the urinary bladder occurred in dosed male rats with a statistically significant positive trend ($P<0.05$; incidence: control, 0/49, 0%; low-dose, 2/49, 4%; high-dose, 4/49, 8%). This benign urinary bladder tumor has not been observed among 568 untreated male control F344/N rats at this laboratory. The incidence of transitional-cell papillomas in male vehicle control rats in all laboratories in the NCI/NTP Bioassay Program is 1/994 (0.1%).

Epithelial hyperplasia was also seen at an increased incidence ($P<0.05$) in the urinary bladder of dosed male rats (control, 0/49, 0%; low-dose, 1/49, 2%; high-dose, 6/49, 12%). This hyperplasia did not occur in the animals that had transitional-cell papillomas. No urinary bladder calculi were seen in male rats.

Fibrosarcomas of the subcutaneous tissue occurred in female rats with a statistically significant positive trend ($P<0.05$; incidence: control, 0/50, 0%; low-dose, 0/50, 0%; high-dose, 3/50, 6%). The incidence in the high-dose group was not significant in comparison with the control group, and the evidence for the association of fibrosarcomas with administration of allyl isothiocyanate is considered equivocal. This tumor has been observed in 1/591 (0.2%) of the untreated female control F344/N rats at this laboratory and in 9/999 (0.9%) of the female vehicle control rats in all laboratories in the NCI/NTP Bioassay Program.

Retinopathy and cataract formation occurred at increased incidence in high-dose male rats and in low-dose female rats. This eye toxicity occurred most frequently in animals placed at the top of the racks, a position that gives maximum light exposure. Other chemicals assayed in a similar manner, such as stannous chloride (NTP, 1982), also showed a correlation between eye toxicity and rack position. However, not all NTP bioassays have shown a correlation between rack placement and eye toxicity. From these incidental observations it is not possible to determine whether a causative relationship exists for light exposure, allyl isothiocyanate administration, and eye defects.

Leukemia occurred in dosed male rats with a statistically significant positive trend ($P<0.05$; incidence: control, 2/50, 4%; low-dose, 6/50, 12%; high-dose, 8/50, 16%). The incidence in the high-dose group was significantly higher than that in the controls ($P<0.05$). However, this observed incidence was not statistically different from the historical incidence in male gavage controls in all laboratories in the Bioassay Program (96/999, 10%). No significant increases were observed for leukemia in female rats (7/50, 9/50, 12/50), or for lymphoma in male and female mice. Consequently, this increase is not considered to be the result of allyl isothiocyanate administration.

Survival of control and dosed female mice was comparable but lower than that usually seen at this laboratory, and the decreased survival may have reduced the incidence of late-appearing tumors in these groups. Suppurative inflammation of the peritoneum, uterus, or multiple organs was found in about one third of the female mice that died before the terminal kill, suggesting that an infection may have been a contributing factor to the decreased survival. Mean body weights of high-dose male and female mice were higher than those of controls throughout most of the study, and the animals may have been able to tolerate higher doses of allyl isothiocyanate.

The incidences of liver tumors in dosed male and female mice were not statistically significant. However, cytoplasmic vacuolization in the liver of dosed male mice was related to administration of allyl isothiocyanate (controls, 2/49, 4%; low-dose, 8/49, 16%; high-dose, 13/50, 26%).

The mechanism of action of allyl isothiocyanate is not known. Other unsaturated compounds, such as haloolefins, are thought to be metabolized *in vivo* to active epoxides (Eder et al., 1980). It

has been suggested that some haloolefins containing an allylic group may act as alkylating agents (Eder et al., 1980). Thiocyanate, which may be metabolically derived from isothiocyanate (White et al., 1978), has been shown to promote nitrosation of amines (Edwards et al., 1979; Fan and Tannenbaum, 1973). Isothiocyanates can react with an alcohol or an amine to give a thiocarbamate or thiourea (March, 1977). It is not known if any of these reactions were involved in producing the "ultimate carcinogen." An alternative mechanism of action for allyl isothiocyanate is as a promoter (Pitot and Sirica, 1980). Allyl isothiocyanate might enhance or stimulate the neoplastic growth of cells already initiated in the bladder cells, rather than initiate the first alteration itself. Allyl isothiocyanate was not mutagenic with or without activation in the Ames assay using strains TA 98, 100, 1535, and 1537 (NTP, 1981).

Other studies have shown that allyl isothiocyanate increases urine excretion (Muztar et al., 1979b). Williams (1974) has shown that allyl isothiocyanate and other isothiocyanates are directly toxic to cells grown in culture. These other toxic effects of allyl isothiocyanate were not measured in this bioassay. Whether they have an association with the carcinogenic effect observed in this study is not known.

Conclusions: Under the conditions of this bioassay, allyl isothiocyanate was carcinogenic for male F344/N rats, causing transitional-cell papillomas of the urinary bladder. Evidence for associating allyl isothiocyanate with subcutaneous fibrosarcomas in female F344/N rats was equivocal. Allyl isothiocyanate was not carcinogenic for B6C3F1 mice of either sex.

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V. REFERENCES

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Armitage, P., Statistical methods in medical research. New York: John Wiley & Sons, Inc.; 1971:362-365.

Berenblum, I. ed., Carcinogencity testing: a report of the panel on carcinogenicity of the Cancer Research Commission of UICC. Geneva: International Union Against Cancer, Vol. 2; 1969.

Cox, D.R., Regression models and life tables. J.R. Stat. Soc. B34; 187-220; 1972.

Eder, E.; Neudecker, T.; Lutz, D.; Henschler, D., Mutagenic potential of allyl and allylic compounds. Biochem. Pharmacol. 29:993-998; 1980.

Edwards, G.; Whong, W-Z.; Speciner, N., Intrahepatic mutagenesis assay: a sensitive method for detecting n-nitrosomorpholine and *in vivo* nitrosation of morpholine. Mutat. Res. 64:415-423; 1979.

Fan, T-Y.; Tannenbaum, S., Factors influencing the rate of formation of nitrosomorpholine from morpholine and nitrite: acceleration by thiocyanate and other anions. J. Agric. Food Chem. 21:237-240; 1973.

Food Chemicals Codex, 1972: 31-32.

Gart, J.; Chu, K.; Tarone, R., Statistical issues in interpretation of chronic bioassay tests for carcinogenicity. J. Natl. Cancer Inst. 62:957; 1979.

Hagan, E., Hansen, W.; Fitzhugh, O.; Jenner, P.; Jones, W.; Taylor, J.; Long, E.; Nelson, A.; Brouwer, J., Food flavourings and compounds of related structure. II. Subacute and chronic toxicity. Food Cosmet. Toxicol. 5:141; 1967.

Hall, R., Toxicants occurring naturally in spices and flavors. In: Toxicants occuring naturally in foods. Washington, DC: National Academy of Science; 1973:448-451.

Jenner, P.; Hagan E.; Taylor, J.; Cook, E.; Fitzhugh, O., Food flavourings and compounds of related structure. I. Acute oral toxicity. Food Cosmet. Toxicol. 2:327-343; 1964.

Kaplan, E.; Meier, P., Nonparametric estimation from incomplete observations. J. Amer. Stat. Assoc. 53:457-481; 1958.

Kirk-Othmer encyclopedia of chemical technology. New York: Interscience Publishers; Vol. 8, 1965:450.

Kirk-Othmer encyclopedia of chemical technology. New York; Interscience Publishers; Vol. 9, 1966:356.

Kirk-Othmer encyclopedia of chemical technology. New York: Interscience Publishers, Vol. 10, 1980:473.

Klesse, P.; Lukoschek, P., Untersuchungen ueber die bakteriostatische Wirksamkeit einiger Senfoele. Arzneim. Forsch. 5:505-507; 1955.

Langer, P.; Greer, M., Antithyroid activity of some naturally occurring isothiocyanates *in vitro*. Metabolism 17:569-605; 1968.

Langer, P.; Stole, V., Goitrogenic activity of allyl isothiocyanate — a widespread natural oil. Endocrinology 76:151-155; 1965.

Life Sciences Research Office, Evaluation of the health aspects of mustard and oil of mustard as food ingredients. Bethesda, MD: Life Sciences Research Office. SCOGS-16, 1975.

Linhart, M.; Cooper, J.; Martin, R.; Page, N.; Peters, J., Carcinogenesis bioassay data system. Comp. Biomed. Res. 7:230-248; 1974.

Mantel, N.; Haenszel, W., Statistical aspects of the analysis of data from retrospective studies of disease. J. Nat. Cancer Inst. 22:719-748; 1959.

March, J., Advanced organic chemistry. New York: McGraw-Hill Book Co.; 1977: 813, 823.

Merck Index, 9th ed. Rahway, NJ: Merck and Co.; 1976: 292.

Mitchell, J.; Jordan, W., Allergic contact dermatitis from the radish *Raphanus Sativus*. Br. J. Dermat. 91:183-189; 1974.

Muztar, A.; Ahmad, P.; Huque, T.; Slinger, S., A study of the chemical binding of allyl isothiocyanate with thyroxine and of the effect of allyl isothiocyanate on lipid metabolism in the rat. Can. J. Physiol. Pharmacol. 57:385-389; 1979a.

Muztar, A.; Huque, T.; Ahmad, P.; Slinger, S., Effect of allyl isothiocyanate on plasma and urinary concentrations of some biochemical entities in the rat. Can. J. Physiol. Pharmacol. 57:504-509; 1979b.

Nishie, K.; Daxenbichler, M., Toxicology of glucosinolates, related compounds (nitriles, R-goitrin, isothiocyanates) and vitamin U found in cruciferae. Food Cosmet. Toxicol. 18:159-172; 1980.

NTIS, National Technical Information Service, PB Report 221 215. GRAS (generally recognized as safe) food ingredients—oil of mustard and allyl isothiocyanate. Maspeth, NY: Food and Drug Research Labs., Inc.; FDABF GRAS 015; October 1972.

V. REFERENCES

NTIS, National Technical Information Service, PB Report 223 812. Teratogenic evaluation of FDA 71-26 (oil of mustard). Maspeth, NY: Food and Drug Research Labs., Inc.; FDABF GRAS, FDA 17-260; June 1973.

NTIS, National Technical Information Service, PB Report 254 528. Evaluation of the health aspects of mustard and oil of mustard as food ingredients. Federation of American Societies for Experimental Biology; 1975.

NTP, National Toxicology Program, NTP Technical Bulletin, Issue 5:9, August 1981.

NTP, National Toxicology Program, NTP Technical Report on the carcinogenesis bioassay of stannous chloride, NTP TR 231, Department of Health and Human Services, Research Triangle Park, North Carolina, 1982.

Oda, Y.; Hamano, Y.; Inoue, K.; Yamamoto, H.; Niihara, T.; Kunita, N., Mutagenicity of food flavours in bacteria. Osaka-Furitsu Koshu Eisei Kenkyu Hokoku, Shokuhin eisei hen 9:177-181; 1978.

Peto, R.; Pike, M.; Day, N.; Gray, R.; Lee, P.; Parish, S.; Peto, J.; Richard, S.; Wahrendorf, J., Guidelines for simple, sensitive, significant tests for carcinogenic effects in long-term animal experiments. International Agency for Research Against Cancer. Monographs on the long-term and short-term screening assays for carcinogens: a critical appraisal. Geneva: World Health Organization. Supplement 2; 1980: 311.

Pitot, H.; Sirica, A., The stages of initiation and promotion in hepatocarcinogenesis. Biochem. Biophys. Acta 605:191-215, 1980.

Ruddick, J.; Newsome, W.; Nash, L., Correlation of teratogenicity and molecular structure: ethylene thiourea and related compounds. Teratology 13:263-266; 1976.

Sadtler Research Laboratories, Sadtler Standard Spectra. Philadelphia: Sadtler Research Laboratories; IR No. 1603; UV No. 459; NMR No. 3155.

Tarone, R., Tests for trend in life table analysis. Biometrika 62:679-682; 1975.

Timmermans, M.; Hennault-Roland, J. Chem. Phys. 29:564-565; 1922.

U.S. CFR, U.S. Code of Federal Regulations, 21CFR:172, 515; 1979.

USITC, United States International Trade Commission, Synthetic organic chemicals: United States production and sales. Washington, DC: U.S. Government Printing Office; 1979; USITC Publication No. 1001.

Vernot, E.; MacEwen, J.; Haun, C.; Kinkead, E., Acute toxicity and skin corrosion data for some organic and inorganic compounds and aqueous solutions. Toxicol. Appl. Pharmacol. 42:417-423; 1977.

Ward, J.; Goodman, D.; Griesemer, R.; Hardisty, J.; Schueler, R.; Squire, R.; Strandberg, J., Quality assurance for pathology in rodent carcinogenesis tests. J. Environ. Path. Toxicol. 2:371-378; 1978.

White, A.; Handler, P.; Smith, E.; Hill, R.; Lehman, I., eds., Principles of biochemistry. New York: McGraw-Hill Book Co.; 1978: 1208.

Williams, G., Direct toxicity of alpha-naphthylisothiocyanate in cell culture. Chem-Biol. Interact. 8:363-369; 1974.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS ADMINISTERED ALLYL ISOTHIOCYANATE BY GAVAGE

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS ADMINISTERED ALLYL ISOTHIOCYANATE IN CORN OIL BY GAVAGE

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(50)
PAPILLOMA, NOS			1 (2%)
SQUAMOUS CELL PAPILLOMA	3 (6%)		4 (8%)
SQUAMOUS CELL CARCINOMA	1 (2%)		2 (4%)
BASAL-CELL TUMOR	1 (2%)		
BASAL-CELL CARCINOMA		1 (2%)	
ADNEXAL ADENOMA	1 (2%)		
KERATOACANTHOMA		1 (2%)	
*SUBCUT TISSUE	(50)	(50)	(50)
SARCOMA, NOS	1 (2%)	3 (6%)	1 (2%)
FIBROMA	2 (4%)	2 (4%)	2 (4%)
FIBROSARCOMA	5 (10%)	5 (10%)	1 (2%)
FIBROUS HISTIOCYTOMA, MALIGNANT	1 (2%)	2 (4%)	
RESPIRATORY SYSTEM			
#LUNG	(49)	(49)	(48)
SQUAMOUS CELL CARCINOMA, UNC PRI			1 (2%)
ALVEOLAR/BRONCHIOLAR ADENOMA	2 (4%)	2 (4%)	1 (2%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (2%)		2 (4%)
SARCOMA, NOS, UNC PRIM OR META			1 (2%)
FIBROSARCOMA, METASTATIC		1 (2%)	
FIBROUS HISTIOCYTOMA, METASTATIC		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
UNDIFFERENTIATED LEUKEMIA	2 (4%)	6 (12%)	8 (16%)
#SPLEEN	(50)	(49)	(50)
HISTIOCYTOMA, METASTATIC		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
CIRCULATORY SYSTEM			
#SPLEEN	(50)	(49)	(50)
HEMANGIOSARCOMA		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(50)	(50)	(50)
NEOPLASTIC NODULE	2 (4%)		5 (10%)
#PANCREAS	(50)	(50)	(49)
ADENOMA, NOS	1 (2%)		
#DUODENUM	(48)	(49)	(47)
MUCINOUS ADENOCARCINOMA			1 (2%)
#ILEUM	(48)	(49)	(47)
OSTEOSARCOMA			1 (2%)
URINARY SYSTEM			
#KIDNEY	(50)	(50)	(50)
TUBULAR-CELL ADENOMA		1 (2%)	
#URINARY BLADDER	(49)	(49)	(49)
TRANSITIONAL-CELL PAPILLOMA		2 (4%)	4 (8%)
LIPOMA		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(47)	(49)	(49)
CARCINOMA,NOS		1 (2%)	
ADENOMA, NOS	7 (15%)	12 (24%)	4 (8%)
#ADRENAL	(50)	(50)	(50)
CORTICAL ADENOMA	1 (2%)		
PHEOCHROMOCYTOMA	16 (32%)	15 (30%)	11 (22%)
PHEOCHROMOCYTOMA, MALIGNANT	1 (2%)	1 (2%)	
GANGLIONEUROMA		1 (2%)	
#THYROID	(48)	(50)	(50)
FOLLICULAR-CELL CARCINOMA	1 (2%)		1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
C-CELL ADENOMA	6 (13%)	10 (20%)	5 (10%)
C-CELL CARCINOMA	2 (4%)	1 (2%)	2 (4%)
#PANCREATIC ISLETS	(50)	(50)	(49)
ISLET-CELL ADENOMA	2 (4%)	2 (4%)	1 (2%)
ISLET-CELL CARCINOMA	1 (2%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
FIBROADENOMA	3 (6%)	3 (6%)	3 (6%)
*PREPUTIAL GLAND	(50)	(50)	(50)
CARCINOMA,NOS		1 (2%)	1 (2%)
ADENOMA, NOS			
ADENOCARCINOMA, NOS	4 (8%)	1 (2%)	1 (2%)
CYSTADENOMA, NOS		1 (2%)	
#TESTIS	(50)	(50)	(49)
INTERSTITIAL-CELL TUMOR	45 (90%)	45 (90%)	49 (100%)
NERVOUS SYSTEM			
#BRAIN	(50)	(49)	(50)
GLIOMA, NOS			1 (2%)
ASTROCYTOMA	2 (4%)		
SPECIAL SENSE ORGANS			
*ZYMBAL'S GLAND	(50)	(50)	(50)
ADENOMA, NOS			1 (2%)
MUSCULOSKELETAL SYSTEM			
*SKULL	(50)	(50)	(50)
OSTEOMA		1 (2%)	
BODY CAVITIES			
*THORAX	(50)	(50)	(50)
ALVEOLAR/BRONCHIOLAR CA, METASTA	1 (2%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
*ABDOMINAL WALL	(50)	(50)	(50)
OSTEOSARCOMA		1 (2%)	
*MESENTERY	(50)	(50)	(50)
MESOTHELIOMA, NOS		1 (2%)	
*TUNICA VAGINALIS	(50)	(50)	(50)
MESOTHELIOMA, NOS			1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(50)	(50)
ALVEOLAR/BRONCHIOLAR CA, METASTA			1 (2%)
SARCOMA, NOS			1 (2%)
FIBROUS HISTIOCYTOMA, METASTATIC	1 (2%)		
MESOTHELIOMA, NOS		1 (2%)	
MESOTHELIOMA, MALIGNANT		1 (2%)	1 (2%)
TAIL			
OSTEOSARCOMA		1	
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	3	4	7
MORIBUND SACRIFICE	10	13	9
SCHEDULED SACRIFICE	5		
ACCIDENTALLY KILLED		1	1
TERMINAL SACRIFICE	32	32	33
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	48	50	49
TOTAL PRIMARY TUMORS	114	128	118
TOTAL ANIMALS WITH BENIGN TUMORS	47	49	49
TOTAL BENIGN TUMORS	90	99	86
TOTAL ANIMALS WITH MALIGNANT TUMORS	17	25	21
TOTAL MALIGNANT TUMORS	22	27	24
TOTAL ANIMALS WITH SECONDARY TUMORS#	2	3	1
TOTAL SECONDARY TUMORS	2	3	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	2	2	6
TOTAL UNCERTAIN TUMORS	2	2	6
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			2
TOTAL UNCERTAIN TUMORS			2

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE A2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS ADMINISTERED ALLYL ISOTHIOCYANATE IN CORN OIL BY GAVAGE

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(50)
BASAL-CELL TUMOR	1 (2%)		
SARCOMA, NOS			1 (2%)
*SUBCUT TISSUE	(50)	(50)	(50)
SARCOMA, NOS			
FIBROMA		2 (4%)	
FIBROSARCOMA			3 (6%)
FIBROUS HISTIOCYTOMA, MALIGNANT	1 (2%)		
OSTEOSARCOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(50)	(50)	(50)
ALVEOLAR/BRONCHIOLAR ADENOMA			1 (2%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (2%)		2 (4%)
C-CELL CARCINOMA, METASTATIC	1 (2%)		
FIBROUS HISTIOCYTOMA, METASTATIC	1 (2%)		
CARCINOSARCOMA			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
MALIG.LYMPHOMA, UNDIFFER-TYPE			1 (2%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (2%)		
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
LEUKEMIA,NOS			1 (2%)
UNDIFFERENTIATED LEUKEMIA	7 (14%)	9 (18%)	11 (22%)
#SPLEEN	(50)	(50)	(50)
OSTEOSARCOMA		1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
*TONGUE	(50)	(50)	(50)
SQUAMOUS CELL PAPILLOMA	1 (2%)		
#SALIVARY GLAND	(50)	(50)	(48)
ADENOMA, NOS	1 (2%)		
#LIVER	(50)	(50)	(50)
NEOPLASTIC NODULE			1 (2%)
FIBROUS HISTIOCYTOMA, METASTATIC	1 (2%)		
#PANCREAS	(49)	(49)	(50)
ADENOMA, NOS			1 (2%)
URINARY SYSTEM			
#URINARY BLADDER	(49)	(49)	(50)
TRANSITIONAL-CELL PAPILLOMA			1 (2%)
ENDOMETRIAL STROMAL SARCOMA, MET			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#PITUITARY	(49)	(50)	(50)
CARCINOMA,NOS		3 (6%)	2 (4%)
ADENOMA, NOS	17 (35%)	10 (20%)	13 (26%)
#ADRENAL	(50)	(50)	(50)
CORTICAL ADENOMA	2 (4%)	2 (4%)	2 (4%)
PHEOCHROMOCYTOMA	1 (2%)	2 (4%)	3 (6%)
PHEOCHROMOCYTOMA, MALIGNANT	1 (2%)		
GANGLIONEUROMA	1 (2%)		
#THYROID	(50)	(48)	(50)
FOLLICULAR-CELL CARCINOMA		1 (2%)	
C-CELL ADENOMA	10 (20%)	8 (17%)	6 (12%)
C-CELL CARCINOMA	2 (4%)	2 (4%)	3 (6%)
#PANCREATIC ISLETS	(49)	(49)	(50)
ISLET-CELL ADENOMA	1 (2%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
ADENOCARCINOMA, NOS	1 (2%)		2 (4%)
FIBROADENOMA	8 (16%)	14 (28%)	11 (22%)
*CLITORAL GLAND	(50)	(50)	(50)
ADENOMA, NOS		1 (2%)	
*VAGINA	(50)	(50)	(50)
SARCOMA, NOS			
FIBROMA			1 (2%)
#UTERUS	(50)	(49)	(50)
ADENOCARCINOMA, NOS	1 (2%)		
LEIOMYOMA			1 (2%)
ENDOMETRIAL STROMAL POLYP	14 (28%)	15 (31%)	16 (32%)
ENDOMETRIAL STROMAL SARCOMA	1 (2%)		
#CERVIX UTERI	(50)	(49)	(50)
SARCOMA, NOS			
#OVARY	(50)	(50)	(50)
CARCINOMA,NOS	1 (2%)		
NERVOUS SYSTEM			
#CEREBRAL VENTRICLE	(50)	(50)	(50)
ASTROCYTOMA		1 (2%)	
#BRAIN	(50)	(50)	(50)
ASTROCYTOMA	1 (2%)		
#BRAIN/THALAMUS	(50)	(50)	(50)
GLIOMA, NOS			1 (2%)
SPECIAL SENSE ORGANS			
*ZYMBAL'S GLAND	(50)	(50)	(50)
BASAL-CELL CARCINOMA	1 (2%)		
MUSCULOSKELETAL SYSTEM			
*SKELETAL MUSCLE	(50)	(50)	(50)
LIPOMA	1 (2%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
*MEDIASTINUM	(50)	(50)	(50)
ALVEOLAR/BRONCHIOLAR CA, INVASIV			1 (2%)
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	6	12	5
MORIBUND SACRIFICE	9	7	12
SCHEDULED SACRIFICE	5		
ACCIDENTALLY KILLED		2	
TERMINAL SACRIFICE	30	29	33
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	42	43	42
TOTAL PRIMARY TUMORS	77	72	86
TOTAL ANIMALS WITH BENIGN TUMORS	37	32	33
TOTAL BENIGN TUMORS	58	54	56
TOTAL ANIMALS WITH MALIGNANT TUMORS	17	16	25
TOTAL MALIGNANT TUMORS	19	18	29
TOTAL ANIMALS WITH SECONDARY TUMORS#	2		1
TOTAL SECONDARY TUMORS	3		1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			1
TOTAL UNCERTAIN TUMORS			1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE A3.

INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE 2-YEAR STUDY OF ALLYL ISOTHIOCYANATE

VEHICLE CONTROL

ANIMAL NUMBER	001	002	003	004	005	006	007	008	009	010	011	012	013	014	015	016	017	018	019	020	021	022	023	024	025
WEEKS ON STUDY	104	104	104	104	104	111	107	109	106	108	108	101	108	106	106	052	102	102	103	101	106	095	106	106	106
INTEGUMENTARY SYSTEM																									
SKIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	N	N	+
SQUAMOUS CELL PAPILLOMA																			X						
SQUAMOUS CELL CARCINOMA																									
BASAL-CELL TUMOR																									
ADNEXAL ADENOMA																									
SUBCUTANEOUS TISSUE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	N	N	+
SARCOMA, NOS						X																			
FIBROMA							X																		
FIBROSARCOMA																					X		X		
FIBROUS HISTIOCYTOMA, MALIGNANT										X															
RESPIRATORY SYSTEM																									
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ALVEOLAR/BRONCHIOLAR ADENOMA																									
ALVEOLAR/BRONCHIOLAR CARCINOMA																									
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
THYMUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
CIRCULATORY SYSTEM																									
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NEOPLASTIC NODULE		X																					X		
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GALLBLADDER & COMMON BILE DUCT	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
PANCREAS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ADENOMA, NOS											X														
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SMALL INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
PITUITARY	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ADENOMA, NOS									X										X			X			
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CORTICAL ADENOMA																			X						
PHEOCHROMOCYTOMA				X		X				X	X							X		X	X		X		X
PHEOCHROMOCYTOMA, MALIGNANT																	X								
THYROID	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
FOLLICULAR-CELL CARCINOMA																									
C-CELL ADENOMA										X															
C-CELL CARCINOMA						X																			
PARATHYROID	+	+	-	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
PANCREATIC ISLETS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ISLET-CELL ADENOMA																									
ISLET-CELL CARCINOMA																									
REPRODUCTIVE SYSTEM																									
MAMMARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	N	N	+	+	N	N	+
FIBROADENOMA												X													
TESTIS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
INTERSTITIAL-CELL TUMOR	X	X	X	X	X	X	X	X		X	X	X	X	X	X		X		X	X	X	X	X	X	X
PROSTATE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PREPUTIAL/CLITORAL GLAND	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
ADENOCARCINOMA, NOS			X																					X	
NERVOUS SYSTEM																									
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ASTROCYTOMA																									
BODY CAVITIES																									
PLEURA	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
ALVEOLAR/BRONCHIOLAR CA, METASTAT																									
ALL OTHER SYSTEMS																									
MULTIPLE ORGANS NOS	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
FIBROUS HISTIOCYTOMA, METASTATIC									X									X							
UNDIFFERENTIATED LEUKEMIA																									

+: TISSUE EXAMINED MICROSCOPICALLY
-: REQUIRED TISSUE NOT EXAMINED MICROSCOPICALLY
X: TUMOR INCIDENCE
M: NECROPSY, NO AUTOLYSIS, NO MICROSCOPIC EXAMINATION

: NO TISSUE INFORMATION SUBMITTED
C: NECROPSY, NO HISTOLOGY DUE TO PROTOCOL
A: AUTOLYSIS
M: ANIMAL MISSING
B: NO NECROPSY PERFORMED

TABLE A3. MALE RATS: TUMOR PATHOLOGY (CONTINUED) VEHICLE CONTROL

ANIMAL NUMBER	021	022	023	024	031	032	033	034	035	036	037	038	039	040	041	042	043	044	045	046	047	048	049	050	TOTAL TISSUES TUMORS
WEEKS ON STUDY	104	104	104	104	104	104	103	104	105	104	102	104	105	104	104	104	104	104	104	104	104	104	103	102	
INTEGUMENTARY SYSTEM																									
SKIN	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	N	+	+	+	+	+	+	+	50N
SQUAMOUS CELL PAPILLOMA																			X					X	3
SQUAMOUS CELL CARCINOMA	X																								1
BASAL-CELL TUMOR																							X		1
ADNEXAL ADENOMA																		X							1
SUBCUTANEOUS TISSUE	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	N	+	+	+	+	+	+	+	50N
SARCOMA, NOS																									1
FIBROMA			X																						2
FIBROSARCOMA	X																X							X	5
FIBROUS HISTIOCYTOMA, MALIGNANT																									1
RESPIRATORY SYSTEM																									
LUNGS AND BRONCHI	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ALVEOLAR/BRONCHIOLAR ADENOMA											X												X		2
ALVEOLAR/BRONCHIOLAR CARCINOMA									X																1
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																									
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	48
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
THYMUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
CIRCULATORY SYSTEM																									
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																									
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	49
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
NEOPLASTIC NODULE																									2
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
GALLBLADDER & COMMON BILE DUCT	N	N	N	N	N	N	N	N	N	+	N	N	+	N	N	N	N	N	N	N	N	N	N	N	50N
PANCREAS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ADENOMA, NOS																									1
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	49
SMALL INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	48
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	48
URINARY SYSTEM																									
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	49
ENDOCRINE SYSTEM																									
PITUITARY	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	47
ADENOMA, NOS			X											X					X	X					7
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
CORTICAL ADENOMA																									1
PHEOCHROMOCYTOMA	X			X		X					X		X			X				X					16
PHEOCHROMOCYTOMA, MALIGNANT																									1
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
FOLLICULAR-CELL CARCINOMA																	X								1
C-CELL ADENOMA			X		X					X						X			X						5
C-CELL CARCINOMA																							X		2
PARATHYROID	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	-	+	-	+	+	-	+	+	42
PANCREATIC ISLETS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ISLET-CELL ADENOMA													X							X					2
ISLET-CELL CARCINOMA				X																					1
REPRODUCTIVE SYSTEM																									
MAMMARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	50N
FIBROADENOMA																X	X								3
TESTIS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
INTERSTITIAL-CELL TUMOR	X	X	X	X	X	X	X	X		X	X		X	X	X	X	X	X	X	X	X	X	X	X	43
PROSTATE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	49
PREPUTIAL/CLITORAL GLAND	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50N
ADENOCARCINOMA, NOS																				X			X		4
NERVOUS SYSTEM																									
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ASTROCYTOMA							X										X								2
BODY CAVITIES																									
PLEURA	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50N
ALVEOLAR/BRONCHIOLAR CA, METASTAT									X																1
ALL OTHER SYSTEMS																									
MULTIPLE ORGANS NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50N
FIBROUS HISTIOCYTOMA, METASTATIC																									1
UNDIFFERENTIATED LEUKEMIA							X																		2

N ANIMALS NECROPSIED

+: TISSUE EXAMINED MICROSCOPICALLY
-: REQUIRED TISSUE NOT EXAMINED MICROSCOPICALLY
X: TUMOR INCIDENCE
N: NECROPSY, NO AUTOLYSIS, NO MICROSCOPIC EXAMINATION

: NO TISSUE INFORMATION SUBMITTED
C: NECROPSY, NO HISTOLOGY DUE TO PROTOCOL
A: AUTOLYSIS
M: ANIMAL MISSING
B: NO NECROPSY PERFORMED

TABLE A3.

INDIVIDUAL ANIMAL T JMOR PATHOLOGY OF MALE RATS IN THE 2-YEAR STUI Y OF ALLYL ISOTHIOCYANATE

LOW DOSE

ANIMAL NUMBER	0010	0020	0030	0040	0050	0060	0070	0080	0090	0100	0110	0120	0130	0140	0150	0160	0170	0180	0190	0200	0210	0220	0230	0240	0250
WEEKS ON STUDY	0651	0661	0705	0705	0772	0705	0705	0705	0705	0705	0705	0705	0705	0682	0671	0705	0705	0705	0705	0705	0705	0665	0705	0705	0703
INTEGUMENTARY SYSTEM																									
SKIN	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+
BASAL-CELL CARCINOMA																									
KERATOACANTHOMA																									
SUBCUTANEOUS TISSUE	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+
SARCOMA, NOS											X														
FIBROMA																									
FIBROSARCOMA	X					X									X										
FIBROUS HISTIOCYTOMA, MALIGNANT		X																							
RESPIRATORY SYSTEM																									
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
ALVEOLAR/BRONCHIOLAR ADENOMA																									
FIBROSARCOMA, METASTATIC																									
FIBROUS HISTIOCYTOMA, METASTATIC																									
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
FIBROUS HISTIOCYTOMA, METASTATIC		X																							
HEMANGIOSARCOMA																									
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
THYMUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CIRCULATORY SYSTEM																									
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GALLBLADDER & COMMON BILE DUCT	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	+	N	N	N	N	N	N
PANCREAS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SMALL INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
TUBULAR-CELL ADENOMA																									
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
TRANSITIONAL-CELL PAPILLOMA																								X	
LIPOMA										X															
ENDOCRINE SYSTEM																									
PITUITARY	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CARCINOMA,NOS																									
ADENOMA, NOS																									
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PHEOCHROMOCYTOMA										X		X						X		X	X				X
PHEOCHROMOCYTOMA, MALIGNANT																									
GANGLIONEUROMA																									
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-CELL ADENOMA																				X					X
C-CELL CARCINOMA																									
PARATHYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PANCREATIC ISLETS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ISLET-CELL ADENOMA																									
REPRODUCTIVE SYSTEM																									
MAMMARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	N	+	+	+	+	+	N	+	+	+
FIBROADENOMA																									
TESTIS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
INTERSTITIAL-CELL TUMOR	X	X		X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X		X		X
PROSTATE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
PREPUTIAL/CLITORAL GLAND																									
CARCINOMA,NOS																									
ADENOCARCINOMA, NOS																									
CYSTADENOMA, NOS																									
NERVOUS SYSTEM																									
BRAIN	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
MUSCULOSKELETAL SYSTEM																									
BONE	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
OSTEOMA																									
BODY CAVITIES																									
PERITONEUM	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
OSTEOSARCOMA																									
MESENTERY	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
MESOTHELIOMA, NOS																									
ALL OTHER SYSTEMS																									
MULTIPLE ORGANS NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
MESOTHELIOMA, NOS															X										
MESOTHELIOMA, MALIGNANT					X																				
MALIG.LYMPHOMA, HISTIOCYTIC TYPE														X											
UNDIFFERENTIATED LEUKEMIA							X						X				X								
TAIL																									
OSTEOSARCOMA																									

+: TISSUE EXAMINED MICROSCOPICALLY
-: REQUIRED TISSUE NOT EXAMINED MICROSCOPICALLY
X: TUMOR INCIDENCE
N: NECROPSY, NO AUTOLYSIS, NO MICROSCOPIC EXAMINATION
1: NO TISSUE INFORMATION SUBMITTED
C: NECROPSY, NO HISTOLOGY DUE TO PROTOCOL
A: AUTOLYSIS
M: ANIMAL MISSING
B: NO NECROPSY PERFORMED

TABLE A3. MALE RATS: TUMOR PATHOLOGY (CONTINUED) LOW DOSE

ANIMAL NUMBER	0224	0227	0228	0229	0230	0231	0232	0233	0234	0235	0236	0237	0238	0239	0240	0241	0242	0243	0244	0245	0246	0247	0248	0249	0250	TOTAL TISSUES TUMORS
WEEKS ON STUDY	105	108	104	108	105	105	100	101	105	105	105	105	105	105	105	108	105	105	108	105	101	105	105	101	105	
INTEGUMENTARY SYSTEM																										
SKIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
BASAL-CELL CARCINOMA										X																1
KERATOACANTHOMA																										
SUBCUTANEOUS TISSUE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SARCOMA, NOS	X			X					X																	3
FIBROMA												X														2
FIBROSARCOMA			X					X																		3
FIBROUS HISTIOCYTOMA, MALIGNANT											X															2
RESPIRATORY SYSTEM																										
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ALVEOLAR/BRONCHIOLAR ADENOMA						X									X											1
FIBROSARCOMA, METASTATIC			X																							
FIBROUS HISTIOCYTOMA, METASTATIC																										
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	49
FIBROUS HISTIOCYTOMA, METASTATIC																										1
HEMANGIOSARCOMA																										
LYMPH NODES																										
THYMUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	49
CIRCULATORY SYSTEM																										
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
GALLBLADDER & COMMON BILE DUCT	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50
PANCREAS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SMALL INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
URINARY SYSTEM																										
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
TUBULAR-CELL ADENOMA																										1
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
TRANSITIONAL-CELL PAPILLOMA								X																		2
LIPOMA																										1
ENDOCRINE SYSTEM																										
PITUITARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
CARCINOMA,NOS																										1
ADENOMA, NOS							X				X		X		X					X	X		X			12
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
PHEOCHROMOCYTOMA	X	X											X						X	X	X	X	X		X	15
PHEOCHROMOCYTOMA, MALIGNANT																										1
GANGLIONEUROMA																										
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-CELL ADENOMA		X	X		X			X			X									X	X				X	10
C-CELL CARCINOMA																										1
PARATHYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
PANCREATIC ISLETS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ISLET-CELL ADENOMA												X										X				2
REPRODUCTIVE SYSTEM																										
MAMMARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
FIBROADENOMA		X				X																				1
TESTIS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
INTERSTITIAL-CELL TUMOR	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	45
PROSTATE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
PREPUTIAL/CLITORAL GLAND	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50
CARCINOMA,NOS																	X									1
ADENOCARCINOMA, NOS																									X	1
CYSTADENOMA, NOS																										1
NERVOUS SYSTEM																										
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
MUSCULOSKELETAL SYSTEM																										
BONE	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50
OSTEOMA							X																			1
BODY CAVITIES																										
PERITONEUM	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50
OSTEOSARCOMA																										1
MESENTERY	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50
MESOTHELIOMA, NOS																	X									1
ALL OTHER SYSTEMS																										
MULTIPLE ORGANS NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50
MESOTHELIOMA, NOS																										1
MESOTHELIOMA, MALIGNANT																										1
MALIG.LYMPHOMA, HISTIOCYTIC TYPE																										1
UNDIFFERENTIATED LEUKEMIA			X		X																			X		6
TAIL																										
OSTEOSARCOMA																										

N: ANIMALS NECROPSIED

+: TISSUE EXAMINED MICROSCOPICALLY
-: REQUIRED TISSUE NOT EXAMINED MICROSCOPICALLY
X: TUMOR INCIDENCE
N: NECROPSY, NO AUTOLYSIS, NO MICROSCOPIC EXAMINATION

: NO TISSUE INFORMATION SUBMITTED
C: NECROPSY, NO HISTOLOGY DUE TO PROTOCOL
A: AUTOLYSIS
M: ANIMAL MISSING
B: NO NECROPSY PERFORMED

TABLE A3.

INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE 2-YEAR STUDY OF ALLYL ISOTHIOCYANATE

HIGH DOSE

ANIMAL NUMBER	001	002	003	004	005	006	007	008	009	010	011	012	013	014	015	016	017	018	019	020	021	022	023	024	025
WEEKS ON STUDY	674	708	708	708	704	704	708	704	708	704	705	701	704	704	708	704	708	708	704	708	704	706	701	708	705
INTEGUMENTARY SYSTEM																									
SKIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PAPILLOMA, NOS		X																							
SQUAMOUS CELL PAPILLOMA													X												
SQUAMOUS CELL CARCINOMA		X																							
SUBCUTANEOUS TISSUE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SARCOMA, NOS																									X
FIBROMA							X							X											
FIBROSARCOMA																									
RESPIRATORY SYSTEM																									
LUNGS AND BRONCHI	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SQUAMOUS CELL CARCINOMA, UNC PRIM												X													
ALVEOLAR/BRONCHIOLAR ADENOMA										X															
ALVEOLAR/BRONCHIOLAR CARCINOMA																						X			
SARCOMA, NOS, UNC PRIM OR META														X											
TRACHEA	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
THYMUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CIRCULATORY SYSTEM																									
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NEOPLASTIC NODULE					X								X							X					
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GALLBLADDER & COMMON BILE DUCT	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
PANCREAS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
STOMACH	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SMALL INTESTINE	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
MUCINOUS ADENOCARCINOMA																									
OSTEOSARCOMA																									
LARGE INTESTINE	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY BLADDER	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
TRANSITIONAL-CELL PAPILLOMA													X											X	
ENDOCRINE SYSTEM																									
PITUITARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
ADENOMA, NOS											X										X				
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PHEOCHROMOCYTOMA					X	X						X	X	X						X					
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
FOLLICULAR-CELL CARCINOMA																		X							
C-CELL ADENOMA											X								X					X	
C-CELL CARCINOMA															X						X				
PARATHYROID	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+
PANCREATIC ISLETS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ISLET-CELL ADENOMA																									
REPRODUCTIVE SYSTEM																									
MAMMARY GLAND	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+
FIBROADENOMA							X																		
TESTIS	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
INTERSTITIAL-CELL TUMOR	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
PROSTATE	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PREPUTIAL/CLITORAL GLAND	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
CARCINOMA,NOS												X													
ADENOCARCINOMA, NOS																									
NERVOUS SYSTEM																									
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GLIOMA, NOS																									
SPECIAL SENSE ORGANS																									
ZYMBAL'S GLAND	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
ADENOMA, NOS																								X	
BODY CAVITIES																									
TUNICA VAGINALIS	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
MESOTHELIOMA, NOS																									
ALL OTHER SYSTEMS																									
MULTIPLE ORGANS NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
ALVEOLAR/BRONCHIOLAR CA, METASTAT																									
SARCOMA, NOS																									
MESOTHELIOMA, MALIGNANT																									
UNDIFFERENTIATED LEUKEMIA								X			X		X								X				

+: TISSUE EXAMINED MICROSCOPICALLY
-: REQUIRED TISSUE NOT EXAMINED MICROSCOPICALLY
X: TUMOR INCIDENCE
N: NECROPSY, NO AUTOLYSIS, NO MICROSCOPIC EXAMINATION

I: NO TISSUE INFORMATION SUBMITTED
C: NECROPSY, NO HISTOLOGY DUE TO PROTOCOL
A: AUTOLYSIS
M: ANIMAL MISSING
B: NO NECROPSY PERFORMED

TABLE A3. MALE RATS: TUMOR PATHOLOGY (CONTINUED) HIGH DOSE

ANIMAL NUMBER / WEEKS ON STUDY	026 104	027 104	028 104	029 104	030 104	031 104	032 104	033 104	034 104	035 103	036 104	037 104	038 104	039 104	040 104	041 104	042 104	043 104	044 104	045 104	046 104	047 104	048 104	049 104	050 104	TOTAL TISSUES TUMORS
INTEGUMENTARY SYSTEM																										
SKIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	50N
PAPILLOMA, NOS																										1
SQUAMOUS CELL PAPILLOMA													X	X										X		4
SQUAMOUS CELL CARCINOMA																	X									2
SUBCUTANEOUS TISSUE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	50N
SARCOMA, NOS																										1
FIBROMA																										2
FIBROSARCOMA										X																1
RESPIRATORY SYSTEM																										
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	48
SQUAMOUS CELL CARCINOMA, UNC PRIM																										1
ALVEOLAR/BRONCHIOLAR ADENOMA																										1
ALVEOLAR/BRONCHIOLAR CARCINOMA																	X									2
SARCOMA, NOS, UNC PRIM OR META																										1
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
HEMATOPOIETIC SYSTEM																										
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
THYMUS	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
CIRCULATORY SYSTEM																										
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
NEOPLASTIC NODULE														X											X	5
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
GALLBLADDER & COMMON BILE DUCT	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50N
PANCREAS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	49
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
SMALL INTESTINE	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
MUCINOUS ADENOCARCINOMA															X											1
OSTEOSARCOMA																						X				1
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
URINARY SYSTEM																										
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
TRANSITIONAL-CELL PAPILLOMA																	X						X			4
ENDOCRINE SYSTEM																										
PITUITARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ADENOMA, NOS		X															X									4
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
PHEOCHROMOCYTOMA	X						X							X							X			X		11
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
FOLLICULAR-CELL CARCINOMA																										1
C-CELL ADENOMA														X			X									5
C-CELL CARCINOMA																										2
PARATHYROID	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	45
PANCREATIC ISLETS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	49
ISLET-CELL ADENOMA																							X			1
REPRODUCTIVE SYSTEM																										
MAMMARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	50N
FIBROADENOMA	X						X																			3
TESTIS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
INTERSTITIAL-CELL TUMOR	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	49
PROSTATE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
PREPUTIAL/CLITORAL GLAND	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50N
CARCINOMA,NOS																										1
ADENOCARCINOMA, NOS																							X			1
NERVOUS SYSTEM																										
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
GLIOMA, NOS											X															1
SPECIAL SENSE ORGANS																										
ZYMBAL'S GLAND	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50N
ADENOMA, NOS																										1
BODY CAVITIES																										
TUNICA VAGINALIS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50N
MESOTHELIOMA, NOS				X																						1
ALL OTHER SYSTEMS																										
MULTIPLE ORGANS NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50N
ALVEOLAR/BRONCHIOLAR CA, METASTAT																	X									1
SARCOMA, NOS																			X							1
MESOTHELIOMA, MALIGNANT										X																1
UNDIFFERENTIATED LEUKEMIA			X										X			X					X					8

N ANIMALS NECROPSIED

+: TISSUE EXAMINED MICROSCOPICALLY
-: REQUIRED TISSUE NOT EXAMINED MICROSCOPICALLY
X: TUMOR INCIDENCE
N: NECROPSY, NO AUTOLYSIS, NO MICROSCOPIC EXAMINATION

: NO TISSUE INFORMATION SUBMITTED
C: NECROPSY, NO HISTOLOGY DUE TO PROTOCOL
A: AUTOLYSIS
M: ANIMAL MISSING
S: NO NECROPSY PERFORMED

TABLE A4.

INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE 2-YEAR STUDY OF ALLYL ISOTHIOCYANATE

VEHICLE CONTROL

ANIMAL NUMBER	001	002	003	004	005	006	007	008	009	010	011	012	013	014	015	016	017	018	019	020	021	022	023	024
WEEKS ON STUDY	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104
INTEGUMENTARY SYSTEM																								
SKIN	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+
BASAL-CELL TUMOR																								
SUBCUTANEOUS TISSUE	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+
FIBROUS HISTIOCYTOMA, MALIGNANT																								
RESPIRATORY SYSTEM																								
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ALVEOLAR/BRONCHIOLAR CARCINOMA																		X						
C-CELL CARCINOMA, METASTATIC													X											
FIBROUS HISTIOCYTOMA, METASTATIC																								
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																								
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
THYMUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CIRCULATORY SYSTEM																								
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																								
ORAL CAVITY	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
SQUAMOUS CELL PAPILLOMA																								
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ADENOMA, NOS																								
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
FIBROUS HISTIOCYTOMA, METASTATIC																								
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GALLBLADDER & COMMON BILE DUCT	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
PANCREAS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SMALL INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																								
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																								
PITUITARY	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ADENOMA, NOS			X	X						X	X			X		X	X			X		X		X X
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CORTICAL ADENOMA							X																	
PHEOCHROMOCYTOMA									X															
PHEOCHROMOCYTOMA, MALIGNANT																								
GANGLIONEUROMA																								
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-CELL ADENOMA	X		X	X			X			X													X	X
C-CELL CARCINOMA																								
PARATHYROID	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
PANCREATIC ISLETS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ISLET-CELL ADENOMA																		X						
REPRODUCTIVE SYSTEM																								
MAMMARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+
ADENOCARCINOMA, NOS																X								
FIBROADENOMA			X		X		X																X	
UTERUS			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ADENOCARCINOMA, NOS																								
ENDOMETRIAL STROMAL POLYP							X				X	X	X	X			X							
ENDOMETRIAL STROMAL SARCOMA																								
OVARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CARCINOMA,NOS																								
NERVOUS SYSTEM																								
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ASTROCYTOMA																								
SPECIAL SENSE ORGANS																								
ZYMBAL'S GLAND	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
BASAL-CELL CARCINOMA																								
MUSCULOSKELETAL SYSTEM																								
MUSCLE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LIPOMA				X																				
ALL OTHER SYSTEMS																								
MULTIPLE ORGANS NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE													X											
UNDIFFERENTIATED LEUKEMIA								X												X				

+: TISSUE EXAMINED MICROSCOPICALLY
-: REQUIRED TISSUE NOT EXAMINED MICROSCOPICALLY
X: TUMOR INCIDENCE
N: NECROPSY, NO AUTOLYSIS, NO MICROSCOPIC EXAMINATION

\ : NO TISSUE INFORMATION SUBMITTED
D: NECROPSY, NO HISTOLOGY DUE TO PROTOCOL
A: AUTOLYSIS
M: ANIMAL MISSING
B: NO NECROPSY PERFORMED

TABLE A4. FEMALE RATS: TUMOR PATHOLOGY (CONTINUED) VEHICLE CONTROL

ANIMAL NUMBER / WEEKS ON STUDY	0 2 5 1 8 4	0 2 7 1 0 6	0 2 8 1 0 1	0 2 9 1 0 0	0 3 0 1 0 9	0 3 1 1 0 1	0 3 2 1 0 6	0 3 3 1 0 9	0 3 5 1 0 6	0 3 7 1 0 8	0 3 8 1 0 8	0 3 9 1 0 0	0 3 9 1 0 0	0 4 0 1 0 8	0 4 1 1 0 9	0 4 2 1 0 9	0 4 3 1 0 8	0 4 4 1 0 6	0 4 5 1 0 0	0 4 6 1 0 4	0 4 7 1 0 6	0 4 8 1 0 3	0 4 9 1 0 0	0 5 0 1 0 6	TOTAL TISSUES TUMORS
INTEGUMENTARY SYSTEM																									
SKIN	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50M
BASAL-CELL TUMOR																									
SUBCUTANEOUS TISSUE	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	[illegible]
FIBROUS HISTIOCYTOMA, MALIGNANT				X																					
RESPIRATORY SYSTEM																									
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ALVEOLAR/BRONCHIOLAR CARCINOMA																									1
C-CELL CARCINOMA, METASTATIC																									
FIBROUS HISTIOCYTOMA, METASTATIC																									
TRACHEA																									
HEMATOPOIETIC SYSTEM																									
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
THYMUS	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	49
CIRCULATORY SYSTEM																									
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																									
ORAL CAVITY	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	50M
SQUAMOUS CELL PAPILLOMA																									
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ADENOMA, NOS																									
LIVER																								+	50
FIBROUS HISTIOCYTOMA, METASTATIC																									1
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
GALLBLADDER & COMMON BILE DUCT	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	50M
PANCREAS	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SMALL INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	49
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	49
URINARY SYSTEM																									
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY BLADDER	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ENDOCRINE SYSTEM																									
PITUITARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ADENOMA, NOS						X		X							X						X	X	X		17
ADRENAL																									
CORTICAL ADENOMA																									
PHEOCHROMOCYTOMA																									
PHEOCHROMOCYTOMA, MALIGNANT																									
GANGLIONEUROMA																									
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-CELL ADENOMA															X			X	X						10
C-CELL CARCINOMA																									
PARATHYROID																									
PANCREATIC ISLETS	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ISLET-CELL ADENOMA																									1
REPRODUCTIVE SYSTEM																									
MAMMARY GLAND	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50M
ADENOCARCINOMA, NOS																									1
FIBROADENOMA										X								X			X	X			8
UTERUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ADENOCARCINOMA, NOS														X											1
ENDOMETRIAL STROMAL POLYP	X		X							X	X	X	X					X						X	14
ENDOMETRIAL STROMAL SARCOMA											X														1
OVARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
CARCINOMA,NOS		X																							1
NERVOUS SYSTEM																									
BRAIN																									50
ASTROCYTOMA																									1
SPECIAL SENSE ORGANS																									
ZYMBAL'S GLAND	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	50M
BASAL-CELL CARCINOMA																									
MUSCULOSKELETAL SYSTEM																									
MUSCLE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50M
LIPOMA																									
ALL OTHER SYSTEMS																									
MULTIPLE ORGANS NOS																									
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE																									
PERENNIATED LEUKEMIA																									

M: ANIMALS NECROPSIED

+: TISSUE EXAMINED MICROSCOPICALLY
-: REQUIRED TISSUE NOT EXAMINED MICROSCOPICALLY
X: TUMOR INCIDENCE
N: NECROPSY, NO AUTOLYSIS, NO MICROSCOPIC EXAMINATION
1: NO TISSUE INFORMATION SUBMITTED
C: NECROPSY, NO HISTOLOGY DUE TO PROTOCOL
A: AUTOLYSIS
M: ANIMAL MISSING
B: NO NECROPSY PERFORMED

TABLE A4.

INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE 2-YEAR STUDY OF ALLYL ISOTHIOCYANATE

LOW DOSE

ANIMAL NUMBER	001	002	003	004	005	006	007	008	009	010	011	012	013	014	015	016	017	018	019	020	021	022	023	024	025
WEEKS ON STUDY	085	095	095	097	072	096	095	095	095	095	081	095	096	096	094	096	096	098	091	095	096	096	096	096	085
INTEGUMENTARY SYSTEM																									
SUBCUTANEOUS TISSUE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
FIBROMA																				X					
OSTEOSARCOMA				X																					
RESPIRATORY SYSTEM																									
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
TRACHEA	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
OSTEOSARCOMA				X																					
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
THYMUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CIRCULATORY SYSTEM																									
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GALLBLADDER & COMMON BILE DUCT	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
PANCREAS	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
SMALL INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+
URINARY SYSTEM																									
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY BLADDER	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
PITUITARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CARCINOMA,NOS													X	X											
ADENOMA, NOS																						X	X		
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CORTICAL ADENOMA																								X	
PHEOCHROMOCYTOMA																									
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
FOLLICULAR-CELL CARCINOMA																X									
C-CELL ADENOMA	X	X					X											X							
C-CELL CARCINOMA										X			X												
PARATHYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
REPRODUCTIVE SYSTEM																									
MAMMARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
FIBROADENOMA		X					X	X	X		X									X					X
PREPUTIAL/CLITORAL GLAND	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
ADENOMA, NOS																									
UTERUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOMETRIAL STROMAL POLYP		X	X			X	X		X		X		X					X						X	
OVARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ASTROCYTOMA					X																				
ALL OTHER SYSTEMS																									
MULTIPLE ORGANS NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
UNDIFFERENTIATED LEUKEMIA																			X		X	X			

+: TISSUE EXAMINED MICROSCOPICALLY
-: REQUIRED TISSUE NOT EXAMINED MICROSCOPICALLY
X: TUMOR INCIDENCE
N: NECROPSY, NO AUTOLYSIS, NO MICROSCOPIC EXAMINATION
: NO TISSUE INFORMATION SUBMITTED
C: NECROPSY, NO HISTOLOGY DUE TO PROTOCOL
A: AUTOLYSIS
M: ANIMAL MISSING
B: NO NECROPSY PERFORMED

TABLE A4. FEMALE RATS: TUMOR PATHOLOGY (CONTINUED) LOW DOSE

ANIMAL NUMBER	022	027	028	029	030	031	032	033	034	035	036	037	038	039	040	041	042	043	044	045	046	047	048	049	050	TOTAL TISSUES TUMORS
WEEKS ON STUDY	109	109	110	110	110	108	107	109	110	110	108	110	110	110	110	110	110	110	109	110	110	110	110	108	109	
INTEGUMENTARY SYSTEM																										
SUBCUTANEOUS TISSUE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	50M
FIBROMA					X																					2
OSTEOSARCOMA																										1
RESPIRATORY SYSTEM																										
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	48
HEMATOPOIETIC SYSTEM																										
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
OSTEOSARCOMA																										1
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
THYMUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
CIRCULATORY SYSTEM																										
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
GALLBLADDER & COMMON BILE DUCT	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50M
PANCREAS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
SMALL INTESTINE	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
LARGE INTESTINE	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
URINARY SYSTEM																										
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ENDOCRINE SYSTEM																										
PITUITARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
CARCINOMA, NOS																										3
ADENOMA, NOS	X		X						X			X	X				X	X	X	X						10
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
CORTICAL ADENOMA							X																			2
PHEOCHROMOCYTOMA									X												X					2
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	48
FOLLICULAR-CELL CARCINOMA																										1
C-CELL ADENOMA		X							X			X								X						8
C-CELL CARCINOMA																										2
PARATHYROID	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	-	-	+	+	+	+	-	+	+	+	45
REPRODUCTIVE SYSTEM																										
MAMMARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	50M
FIBROADENOMA							X				X	X	X							X	X			X		14
PREPUTIAL/CLITORAL GLAND	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	50M
ADENOMA, NOS					X																					1
UTERUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	49
ENDOMETRIAL STROMAL POLYP			X									X	X	X								X	X			15
OVARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
NERVOUS SYSTEM																										
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ASTROCYTOMA																										1
ALL OTHER SYSTEMS																										
MULTIPLE ORGANS NOS	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	50M
UNDIFFERENTIATED LEUKEMIA	X	X								X						X		X							X	9

M ANIMALS NECROPSIED

+: TISSUE EXAMINED MICROSCOPICALLY
-: REQUIRED TISSUE NOT EXAMINED MICROSCOPICALLY
X: TUMOR INCIDENCE
M: NECROPSY, NO AUTOLYSIS, NO MICROSCOPIC EXAMINATION

I: NO TISSUE INFORMATION SUBMITTED
C: NECROPSY, NO HISTOLOGY DUE TO PROTOCOL
A: AUTOLYSIS
M: ANIMAL MISSING
B: NO NECROPSY PERFORMED

TABLE A4.

INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE 2-YEAR STUDY OF ALLYL ISOTHIOCYANATE

HIGH DOSE

ANIMAL NUMBER	001	002	003	004	005	006	007	008	009	010	011	012	013	014	015	016	017	018	019	020	021	022	023	024	025
WEEKS ON STUDY	089	104	107	123	084	095	096	096	104	084	092	095	094	092	095	091	095	095	091	095	095	090	095	095	102
INTEGUMENTARY SYSTEM																									
SKIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	N
SARCOMA, NOS																						X			
SUBCUTANEOUS TISSUE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	N
FIBROSARCOMA					X																				
RESPIRATORY SYSTEM																									
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ALVEOLAR/BRONCHIOLAR ADENOMA																			X						
ALVEOLAR/BRONCHIOLAR CARCINOMA												X													
CARCINOSARCOMA																									
TRACHEA	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
THYMUS	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
CIRCULATORY SYSTEM																									
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NEOPLASTIC NODULE																									
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GALLBLADDER & COMMON BILE DUCT	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	+	N	N	N
PANCREAS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ADENOMA, NOS																									
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SMALL INTESTINE	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
TRANSITIONAL-CELL PAPILLOMA																									
ENDOCRINE SYSTEM																									
PITUITARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CARCINOMA, NOS																									
ADENOMA, NOS					X		X				X	X	X		X			X			X				
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CORTICAL ADENOMA											X													X	
PHEOCHROMOCYTOMA																									
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-CELL ADENOMA							X	X			X					X									
C-CELL CARCINOMA																									
PARATHYROID	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
REPRODUCTIVE SYSTEM																									
MAMMARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+
ADENOCARCINOMA, NOS					X							X													
FIBROADENOMA		X											X	X		X			X					X	
VAGINA	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
FIBROMA										X															
UTERUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LEIOMYOMA																		X							
ENDOMETRIAL STROMAL POLYP					X	X		X	X		X			X				X			X				
OVARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GLIOMA, NOS																									
BODY CAVITIES																									
MEDIASTINUM	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
ALVEOLAR/BRONCHIOLAR CA, INVASIV												X													
ALL OTHER SYSTEMS																									
MULTIPLE ORGANS NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
MALIG.LYMPHOMA, UNDIFFER-TYPE			X																						
MALIG.LYMPHOMA, HISTIOCYTIC TYPE				X																					
LEUKEMIA,NOS																									
UNDIFFERENTIATED LEUKEMIA						X		X				X							X						X

+: TISSUE EXAMINED MICROSCOPICALLY
-: REQUIRED TISSUE NOT EXAMINED MICROSCOPICALLY
X: TUMOR INCIDENCE
N: NECROPSY, NO AUTOLYSIS, NO MICROSCOPIC EXAMINATION

!: NO TISSUE INFORMATION SUBMITTED
C: NECROPSY, NO HISTOLOGY DUE TO PROTOCOL
A: AUTOLYSIS
M: ANIMAL MISSING
B: NO NECROPSY PERFORMED

TABLE A4. FEMALE RATS: TUMOR PATHOLOGY (CONTINUED) HIGH DOSE

ANIMAL NUMBER	0122	0127	0128	0129	0130	0131	0132	0133	0134	0135	0136	0137	0138	0139	0140	0141	0142	0143	0144	0145	0146	0147	0148	0149	0150	TOTAL TISSUES TUMORS
WEEKS ON STUDY	0721	0815	0915	0905	1105	0805	1109	0909	1105	0905	1105	0605	1105	0905	1105	0905	1105	0805	1105	0005	1105	0905	0804	0805	1105	
INTEGUMENTARY SYSTEM																										
SKIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	50M
SARCOMA, NOS																										1
SUBCUTANEOUS TISSUE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	50M
FIBROSARCOMA						X																		X		3
RESPIRATORY SYSTEM																										
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ALVEOLAR/BRONCHIOLAR ADENOMA																										1
ALVEOLAR/BRONCHIOLAR CARCINOMA					X																					2
CARCINOSARCOMA																										1
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
HEMATOPOIETIC SYSTEM																										
BONE MARROW	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
THYMUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	47
CIRCULATORY SYSTEM																										
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
NEOPLASTIC NODULE								X																		1
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
GALLBLADDER & COMMON BILE DUCT	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	+	N	N	N	N	N	N	N	N	N	50M
PANCREAS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ADENOMA, NOS						X																				1
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SMALL INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
URINARY SYSTEM																										
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
TRANSITIONAL-CELL PAPILLOMA																X										1
ENDOCRINE SYSTEM																										
PITUITARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
CARCINOMA,NOS																X						X				2
ADENOMA, NOS					X				X		X										X				X	13
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
CORTICAL ADENOMA																										2
PHEOCHROMOCYTOMA				X					X																	3
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-CELL ADENOMA								X																	X	6
C-CELL CARCINOMA		X												X					X							3
PARATHYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	47
REPRODUCTIVE SYSTEM																										
MAMMARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50M
ADENOCARCINOMA, NOS																										2
FIBROADENOMA					X							X	X			X	X									11
VAGINA	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50M
FIBROMA																										1
UTERUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
LEIOMYOMA																										1
ENDOMETRIAL STROMAL POLYP							X	X				X	X	X					X		X			X		16
OVARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
NERVOUS SYSTEM																										
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
GLIOMA, NOS										X																1
BODY CAVITIES																										
MEDIASTINUM	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50M
ALVEOLAR/BRONCHIOLAR CA, INVASIVE																										1
ALL OTHER SYSTEMS																										
MULTIPLE ORGANS NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50M
MALIG.LYMPHOMA, UNDIFFER-TYPE																										1
MALIG.LYMPHOMA, HISTIOCYTIC TYPE																										1
LEUKEMIA,NOS																				X						1
UNDIFFERENTIATED LEUKEMIA	X		X				X				X										X				X	11

M ANIMALS NECROPSIED

+: TISSUE EXAMINED MICROSCOPICALLY
-: REQUIRED TISSUE NOT EXAMINED MICROSCOPICALLY
X: TUMOR INCIDENCE
N: NECROPSY, NO AUTOLYSIS, NO MICROSCOPIC EXAMINATION

I: NO TISSUE INFORMATION SUBMITTED
C: NECROPSY, NO HISTOLOGY DUE TO PROTOCOL
A: AUTOLYSIS
M: ANIMAL MISSING
B: NO NECROPSY PERFORMED

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APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE ADMINISTERED ALLYL ISOTHIOCYANATE BY GAVAGE

TABLE B1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE ADMINISTERED ALLYL ISOTHIOCYANATE IN CORN OIL BY GAVAGE

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(50)
PAPILLOMA, NOS	1 (2%)		
RESPIRATORY SYSTEM			
#LUNG	(50)	(50)	(50)
HEPATOCELLULAR CARCINOMA, METAST	5 (10%)	2 (4%)	
ALVEOLAR/BRONCHIOLAR ADENOMA	4 (8%)	3 (6%)	5 (10%)
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (2%)	3 (6%)
SARCOMA, NOS, METASTATIC			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	2 (4%)		
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (2%)	2 (4%)	
CIRCULATORY SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
HEMANGIOSARCOMA	1 (2%)		1 (2%)
#SPLEEN	(49)	(48)	(50)
HEMANGIOSARCOMA	1 (2%)	1 (2%)	1 (2%)
#MYOCARDIUM	(50)	(50)	(50)
HEMANGIOMA		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(49)	(49)	(50)
BILE DUCT CARCINOMA			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
HEPATOCELLULAR ADENOMA	9 (18%)	6 (12%)	12 (24%)
HEPATOCELLULAR CARCINOMA	13 (27%)	9 (18%)	10 (20%)
MIXED HEPATO/CHOLANGIO CARCINOMA			1 (2%)
#STOMACH	(49)	(48)	(48)
SQUAMOUS CELL CARCINOMA			1 (2%)
#JEJUNUM	(45)	(42)	(45)
CARCINOMA,NOS		1 (2%)	
URINARY SYSTEM			
#KIDNEY/CORTEX	(49)	(49)	(50)
ADENOMA, NOS			
ENDOCRINE SYSTEM			
#ADRENAL	(47)	(49)	(50)
PHEOCHROMOCYTOMA			
#THYROID	(50)	(45)	(50)
FOLLICULAR-CELL ADENOMA	3 (6%)	2 (4%)	1 (2%)
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(50)	(50)	(50)
ADENOMA, NOS	2 (4%)	1 (2%)	1 (2%)
CYSTADENOMA, NOS	1 (2%)		
MUSCULOSKELETAL SYSTEM			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
*MEDIASTINUM	(50)	(50)	(50)
ALVEOLAR/BRONCHIOLAR CA, INVASIV			
ALVEOLAR/BRONCHIOLAR CA, METASTA			1 (2%)
*MESENTERY	(50)	(50)	(50)
MESOTHELIOMA, NOS	1 (2%)		
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(50)	(50)
SQUAMOUS CELL CARCINOMA, METASTA			1 (2%)
HEPATOCELLULAR CARCINOMA, METAST	1 (2%)		
FIBROSARCOMA			1 (2%)
HEAD			
SARCOMA, NOS			1
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	14	17	10
MORIBUND SACRIFICE	9	3	6
SCHEDULED SACRIFICE	5		
ACCIDENTALLY KILLED	1	6	7
TERMINAL SACRIFICE	21	24	27
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	33	22	26
TOTAL PRIMARY TUMORS	39	27	39
TOTAL ANIMALS WITH BENIGN TUMORS	18	12	18
TOTAL BENIGN TUMORS	20	13	19
TOTAL ANIMALS WITH MALIGNANT TUMORS	18	14	17
TOTAL MALIGNANT TUMORS	18	14	20
TOTAL ANIMALS WITH SECONDARY TUMORS#	6	2	3
TOTAL SECONDARY TUMORS	6	2	3
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	1		
TOTAL UNCERTAIN TUMORS	1		
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE B2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE ADMINISTERED ALLYL ISOTHIOCYANATE IN CORN OIL BY GAVAGE

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	49
INTEGUMENTARY SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(49)
FIBROUS HISTIOCYTOMA, MALIGNANT			1 (2%)
*SUBCUT TISSUE	(50)	(50)	(49)
MALIGNANT MELANOMA		1 (2%)	
FIBROUS HISTIOCYTOMA, MALIGNANT			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(47)	(49)	(49)
SQUAMOUS CELL CARCINOMA, METASTA			1 (2%)
ALVEOLAR/BRONCHIOLAR ADENOMA	2 (4%)	1 (2%)	
ALVEOLAR/BRONCHIOLAR CARCINOMA		2 (4%)	3 (6%)
OSTEOSARCOMA, METASTATIC			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(49)
MALIGNANT LYMPHOMA, NOS	1 (2%)	1 (2%)	
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	3 (6%)	2 (4%)	1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (2%)		2 (4%)
MALIGNANT LYMPHOMA, MIXED TYPE			
LYMPHOCYTIC LEUKEMIA			1 (2%)
#SPLEEN	(47)	(48)	(49)
MALIGNANT LYMPHOMA, MIXED TYPE			1 (2%)
#MESENTERIC L. NODE	(50)	(47)	(49)
MALIGNANT LYMPHOMA, MIXED TYPE		1 (2%)	
#LIVER	(50)	(49)	(49)
KUPFFER-CELL SARCOMA			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
UNDIFFERENTIATED LEUKEMIA			1 (2%)
CIRCULATORY SYSTEM			
*SKIN	(50)	(50)	(49)
HEMANGIOMA		1 (2%)	
*SUBCUT TISSUE	(50)	(50)	(49)
HEMANGIOSARCOMA	1 (2%)		
LYMPHANGIOMA			1 (2%)
#SPLEEN	(47)	(48)	(49)
HEMANGIOSARCOMA			1 (2%)
*MESENTERY	(50)	(50)	(49)
HEMANGIOMA		1 (2%)	
#UTERUS	(50)	(47)	(49)
HEMANGIOSARCOMA	1 (2%)		
#OVARY	(49)	(44)	(48)
HEMANGIOSARCOMA		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(50)	(49)	(49)
HEPATOCELLULAR ADENOMA	2 (4%)	1 (2%)	
HEPATOCELLULAR CARCINOMA		2 (4%)	1 (2%)
#STOMACH	(47)	(47)	(49)
SQUAMOUS CELL PAPILLOMA		1 (2%)	
SQUAMOUS CELL CARCINOMA			
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(47)	(45)	(44)
CARCINOMA,NOS	3 (6%)	3 (7%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ADENOMA, NOS	3 (6%)	3 (7%)	4 (9%)
ACIDOPHIL CARCINOMA	1 (2%)		
#THYROID	(48)	(47)	(47)
FOLLICULAR-CELL ADENOMA	1 (2%)	3 (6%)	1 (2%)
FOLLICULAR-CELL CARCINOMA			2 (4%)
#PANCREATIC ISLETS	(47)	(45)	(49)
ISLET-CELL ADENOMA		1 (2%)	
ISLET-CELL CARCINOMA			
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(49)
ADENOMA, NOS	1 (2%)		
ADENOCARCINOMA, NOS	1 (2%)	1 (2%)	1 (2%)
#UTERUS	(50)	(47)	(49)
SQUAMOUS CELL CARCINOMA			1 (2%)
ADENOCARCINOMA, NOS		1 (2%)	
ENDOMETRIAL STROMAL POLYP	2 (4%)		
#OVARY	(49)	(44)	(48)
TERATOMA, NOS			1 (2%)
NERVOUS SYSTEM			
#BRAIN	(50)	(50)	(49)
ACIDOPHIL CARCINOMA, INVASIVE	1 (2%)		
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(50)	(50)	(49)
ADENOMA, NOS	1 (2%)	1 (2%)	
CYSTADENOMA, NOS	1 (2%)		
MUSCULOSKELETAL SYSTEM			
*FEMUR	(50)	(50)	(49)
OSTEOSARCOMA			1 (2%)
BODY CAVITIES			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	22	15	16
MORIBUND SACRIFICE	12	10	15
SCHEDULED SACRIFICE	5		
ACCIDENTALLY KILLED			1
TERMINAL SACRIFICE	11	25	18
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	18	20	20
TOTAL PRIMARY TUMORS	25	28	26
TOTAL ANIMALS WITH BENIGN TUMORS	11	11	6
TOTAL BENIGN TUMORS	13	13	6
TOTAL ANIMALS WITH MALIGNANT TUMORS	10	14	15
TOTAL MALIGNANT TUMORS	12	15	19
TOTAL ANIMALS WITH SECONDARY TUMORS#	1		2
TOTAL SECONDARY TUMORS	1		2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			1
TOTAL UNCERTAIN TUMORS			1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE B3.

INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE 2-YEAR STUDY OF ALLYL ISOTHIOCYANATE

VEHICLE CONTROL

ANIMAL NUMBER	001	002	003	004	005	006	007	008	009	010	011	012	013	014	015	016	017	018	019	020	021	022	023	024
WEEKS ON STUDY	099	082	087	084	081	084	091	081	085	086	078	085	079	084	081	080	089	081	086	081	082	083	082	084
INTEGUMENTARY SYSTEM																								
SKIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PAPILLOMA, NOS								X																
RESPIRATORY SYSTEM																								
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEPATOCELLULAR CARCINOMA, METASTA	X																					X	X	
ALVEOLAR/BRONCHIOLAR ADENOMA						X																		
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																								
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMANGIOSARCOMA										X														
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
THYMUS	+	-	-	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
CIRCULATORY SYSTEM																								
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																								
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
HEPATOCELLULAR ADENOMA										X														X
HEPATOCELLULAR CARCINOMA	X	X	X		X												X					X	X	
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
GALLBLADDER & COMMON BILE DUCT	N	N	+	+	+	+	+	+	+	+	N	+	+	+	+	N	N	+	N	+	+	+	N	+
PANCREAS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
SMALL INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
URINARY SYSTEM																								
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																								
PITUITARY	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
ADRENAL	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
FOLLICULAR-CELL ADENOMA						X									X			X						
PARATHYROID	+	+	+	+	+	+	+	+	-	+	-	+	+	-	+	+	+	+	-	+	+	+	+	+
REPRODUCTIVE SYSTEM																								
MAMMARY GLAND	N	N	+	N	N	N	N	N	N	N	N	N	N	+	N	N	N	N	N	N	N	N	+	+
TESTIS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PROSTATE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																								
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																								
HARDERIAN GLAND	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
ADENOMA, NOS																				X				
CYSTADENOMA, NOS																			X					
BODY CAVITIES																								
MESENTERY	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
MESOTHELIOMA, NOS																	X							
ALL OTHER SYSTEMS																								
MULTIPLE ORGANS NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
HEPATOCELLULAR CARCINOMA, METASTA																	X							
HEMANGIOSARCOMA																			X					
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE																								
MALIG.LYMPHOMA, HISTIOCYTIC TYPE																								

+: TISSUE EXAMINED MICROSCOPICALLY
-: REQUIRED TISSUE NOT EXAMINED MICROSCOPICALLY
X: TUMOR INCIDENCE
N: NECROPSY, NO AUTOLYSIS, NO MICROSCOPIC EXAMINATION

: NO TISSUE INFORMATION SUBMITTED
C: NECROPSY, NO HISTOLOGY DUE TO PROTOCOL
A: AUTOLYSIS
M: ANIMAL MISSING
B: NO NECROPSY PERFORMED

TABLE B3. MALE MICE: TUMOR PATHOLOGY (CONTINUED) VEHICLE CONTROL

ANIMAL NUMBER	026	027	028	029	030	031	032	033	034	035	036	037	038	039	040	041	042	043	044	045	046	047	048	049	050	TOTAL TISSUES TUMORS
WEEKS ON STUDY	057	106	106	106	104	075	091	076	106	093	078	106	091	106	106	106	066	041	106	083	096	106	063	106	106	
INTEGUMENTARY SYSTEM																										
SKIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50*
PAPILLOMA, NOS																										1
RESPIRATORY SYSTEM																										
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEPATOCELLULAR CARCINOMA, METASTA										X	X															5
ALVEOLAR/BRONCHIOLAR ADENOMA				X										X										X		4
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	49
HEMANGIOSARCOMA																										1
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
THYMUS	-	+	+	+	+	-	+	+	+	-	-	+	+	+	+	+	+	+	+	+	-	+	+	+	+	41
CIRCULATORY SYSTEM																										
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
HEPATOCELLULAR ADENOMA			X		X		X		X					X	X	X										9
HEPATOCELLULAR CARCINOMA						X				X	X		X		X				X							13
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
GALLBLADDER & COMMON BILE DUCT	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	N	+	+	50*
PANCREAS	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	47
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
SMALL INTESTINE	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	45
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
URINARY SYSTEM																										
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ENDOCRINE SYSTEM																										
PITUITARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	46
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	47
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
FOLLICULAR-CELL ADENOMA																										1
PARATHYROID	+	-	+	+	-	+	+	+	+	+	+	-	+	+	+	-	-	-	+	+	+	+	+	+	+	40
REPRODUCTIVE SYSTEM																										
MAMMARY GLAND	N	+	N	N	N	N	N	N	N	+	N	N	+	N	N	N	N	N	N	N	N	N	N	N	N	50*
TESTIS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
PROSTATE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
NERVOUS SYSTEM																										
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSE ORGANS																										
HARDERIAN GLAND	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50*
ADENOMA, NOS																						X				2
CYSTADENOMA, NOS																										1
BODY CAVITIES																										
MESENTERY	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50*
MESOTHELIOMA, NOS																										1
ALL OTHER SYSTEMS																										
MULTIPLE ORGANS NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50*
HEPATOCELLULAR CARCINOMA, METASTA																										1
HEMANGIOSARCOMA																										1
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE												X									X					2
MALIG.LYMPHOMA, HISTIOCYTIC TYPE								X																		1

* ANIMALS NECROPSIED

+: TISSUE EXAMINED MICROSCOPICALLY
-: REQUIRED TISSUE NOT EXAMINED MICROSCOPICALLY
X: TUMOR INCIDENCE
N: NECROPSY, NO AUTOLYSIS, NO MICROSCOPIC EXAMINATION
: NO TISSUE INFORMATION SUBMITTED
C: NECROPSY, NO HISTOLOGY DUE TO PROTOCOL
A: AUTOLYSIS
M: ANIMAL MISSING
B: NO NECROPSY PERFORMED

TABLE B3.

INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE 2-YEAR STUDY OF ALLYL ISOTHIOCYANATE

LOW DOSE

ANIMAL NUMBER	001	002	003	004	005	006	007	008	009	010	011	012	013	014	015	016	017	018	019	020	021	022	023	024	025
WEEKS ON STUDY	012	115	105	105	027	105	105	105	105	075	086	105	061	052	065	055	097	105	103	060	099	105	097	021	011
RESPIRATORY SYSTEM																									
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEPATOCELLULAR CARCINOMA, METASTA																				X					
ALVEOLAR/BRONCHIOLAR ADENOMA									X							X									
ALVEOLAR/BRONCHIOLAR CARCINOMA																									
TRACHEA	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
BONE MARROW	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPLEEN	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMANGIOSARCOMA																	X								
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
THYMUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
CIRCULATORY SYSTEM																									
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMANGIOMA												X													
DIGESTIVE SYSTEM																									
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LIVER	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEPATOCELLULAR ADENOMA			X							X		X													
HEPATOCELLULAR CARCINOMA																X			X	X					
BILE DUCT	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GALLBLADDER & COMMON BILE DUCT	+	+	+	+	N	+	+	+	+	+	N	+	+	N	+	+	N	N	+	N	+	+	+	+	N
PANCREAS	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
STOMACH	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SMALL INTESTINE	+	+	+	+	-	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-
CARCINOMA,NOS															X										
LARGE INTESTINE	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
KIDNEY	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
PITUITARY	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	+	+	+	+
ADRENAL	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
THYROID	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-
FOLLICULAR-CELL ADENOMA																				X					
PARATHYROID	-	-	-	-	+	-	+	+	-	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	-
REPRODUCTIVE SYSTEM																									
MAMMARY GLAND	N	N	+	N	N	+	N	N	N	N	N	N	N	+	N	+	N	N	N	+	N	N	N	N	N
TESTIS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PROSTATE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																									
HARDERIAN GLAND	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
ADENOMA, NOS																									
ALL OTHER SYSTEMS																									
MULTIPLE ORGANS NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
MALIG.LYMPHOMA, HISTIOCYTIC TYPE						X										X									

+: TISSUE EXAMINED MICROSCOPICALLY
-: REQUIRED TISSUE NOT EXAMINED MICROSCOPICALLY
X: TUMOR INCIDENCE
N: NECROPSY, NO AUTOLYSIS, NO MICROSCOPIC EXAMINATION

: NO TISSUE INFORMATION SUBMITTED
C: NECROPSY, NO HISTOLOGY DUE TO PROTOCOL
A: AUTOLYSIS
M: ANIMAL MISSING
B: NO NECROPSY PERFORMED

TABLE B3. MALE MICE: TUMOR PATHOLOGY (CONTINUED) LOW DOSE

ANIMAL NUMBER	0226	0227	0228	0229	0301	0302	0303	0304	0305	0306	0307	0308	0309	0310	0401	0402	0403	0404	0405	0406	0407	0408	0409	0410	TOTAL TISSUES TUMORS
WEEKS ON STUDY	103	109	76	93	105	108	105	105	104	102	105	102	105	105	104	101	108	109	105	105	105	104	105	105	
RESPIRATORY SYSTEM																									
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEPATOCELLULAR CARCINOMA, METASTA														X											2
ALVEOLAR/BRONCHIOLAR ADENOMA	X																								3
ALVEOLAR/BRONCHIOLAR CARCINOMA											X														1
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
HEMATOPOIETIC SYSTEM																									
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
SPLEEN	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
HEMANGIOSARCOMA																									1
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
THYMUS	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
CIRCULATORY SYSTEM																									
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMANGIOMA																									1
DIGESTIVE SYSTEM																									
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
HEPATOCELLULAR ADENOMA					X																X				6
HEPATOCELLULAR CARCINOMA								X				X		X					X	X			X	X	9
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
GALLBLADDER & COMMON BILE DUCT	+	+	N	N	+	+	N	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	50N
PANCREAS	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	47
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
STOMACH	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
SMALL INTESTINE	+	+	-	-	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	42
CARCINOMA,NOS																									1
LARGE INTESTINE	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
URINARY SYSTEM																									
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
URINARY BLADDER	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
ENDOCRINE SYSTEM																									
PITUITARY	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
THYROID	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
FOLLICULAR-CELL ADENOMA																			X						2
PARATHYROID	+	+	-	-	+	+	-	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	36
REPRODUCTIVE SYSTEM																									
MAMMARY GLAND	N	N	N	N	N	N	N	N	N	N	N	+	+	N	N	N	+	N	N	N	N	+	N	+	N	50N
TESTIS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
PROSTATE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
NERVOUS SYSTEM																									
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSE ORGANS																									
HARDERIAN GLAND	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50N
ADENOMA, NOS																								X	1
ALL OTHER SYSTEMS																									
MULTIPLE ORGANS NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50N
MALIG.LYMPHOMA, HISTIOCYTIC TYPE																									2

N ANIMALS NECROPSIED

+: TISSUE EXAMINED MICROSCOPICALLY
-: REQUIRED TISSUE NOT EXAMINED MICROSCOPICALLY
X: TUMOR INCIDENCE
N: NECROPSY, NO AUTOLYSIS, NO MICROSCOPIC EXAMINATION
T: NO TISSUE INFORMATION SUBMITTED
C: NECROPSY, NO HISTOLOGY DUE TO PROTOCOL
A: AUTOLYSIS
M: ANIMAL MISSING
B: NO NECROPSY PERFORMED

TABLE B3.

INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE 2-YEAR STUDY OF ALLYL ISOTHIOCYANATE

HIGH DOSE

ANIMAL NUMBER	001	002	003	004	005	006	007	008	009	010	011	012	013	014	015	016	017	018	019	020	021	022	023	024	025
WEEKS ON STUDY	104	104	105	106	104	105	105	104	104	104	104	102	104	105	106	104	105	106	107	104	105	104	104	104	104
RESPIRATORY SYSTEM																									
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ALVEOLAR/BRONCHIOLAR ADENOMA																									
ALVEOLAR/BRONCHIOLAR CARCINOMA										X															
SARCOMA, NOS, METASTATIC																									
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMANGIOSARCOMA																									
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
THYMUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CIRCULATORY SYSTEM																									
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
BILE DUCT CARCINOMA																									
HEPATOCELLULAR ADENOMA						X				X													X	X	X
HEPATOCELLULAR CARCINOMA	X					X		X			X		X			X	X						X		
MIXED HEPATO/CHOLANGIO CARCINOMA																									
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GALLBLADDER & COMMON BILE DUCT	+	+	N	N	N	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+
PANCREAS	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SQUAMOUS CELL CARCINOMA																									
SMALL INTESTINE	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
PITUITARY	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
FOLLICULAR-CELL ADENOMA													X												
PARATHYROID	+	-	+	+	-	-	-	+	+	+	+	+	+	+	-	-	+	+	-	+	+	+	+	-	+
REPRODUCTIVE SYSTEM																									
MAMMARY GLAND	N	N	N	N	N	N	N	N	N	N	N	+	N	N	N	N	N	+	N	N	+	N	N	N	N
TESTIS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PROSTATE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																									
HARDERIAN GLAND	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
ADENOMA, NOS	X																								
BODY CAVITIES																									
MEDIASTINUM	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
ALVEOLAR/BRONCHIOLAR CA, METASTAT																									
ALL OTHER SYSTEMS																									
MULTIPLE ORGANS NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
SQUAMOUS CELL CARCINOMA, METASTAT																									
FIBROSARCOMA																									
HEMANGIOSARCOMA																									
HEAD NOS																									
SARCOMA, NOS																									

+: TISSUE EXAMINED MICROSCOPICALLY
-: REQUIRED TISSUE NOT EXAMINED MICROSCOPICALLY
X: TUMOR INCIDENCE
N: NECROPSY, NO AUTOLYSIS, NO MICROSCOPIC EXAMINATION
: NO TISSUE INFORMATION SUBMITTED
C: NECROPSY, NO HISTOLOGY DUE TO PROTOCOL
A: AUTOLYSIS
M: ANIMAL MISSING
B: NO NECROPSY PERFORMED

TABLE B3. MALE MICE: TUMOR PATHOLOGY (CONTINUED) HIGH DOSE

ANIMAL NUMBER	002	002	002	002	003	003	003	003	003	003	003	003	003	004	004	004	004	004	004	004	004	004	004	005	TOTAL TISSUES TUMORS
WEEKS ON STUDY	044	053	058	059	060	061	062	063	064	065	066	067	068	069	070	071	072	073	074	075	076	077	078	079	
RESPIRATORY SYSTEM																									
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ALVEOLAR/BRONCHIOLAR ADENOMA		X											X					X				X			5
ALVEOLAR/BRONCHIOLAR CARCINOMA		X				X																		X	3
SARCOMA, NOS, METASTATIC									X																1
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
HEMATOPOIETIC SYSTEM																									
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMANGIOSARCOMA																X									1
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
THYMUS	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	-	+	+	+	46
CIRCULATORY SYSTEM																									
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																									
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
BILE DUCT CARCINOMA																		X							1
HEPATOCELLULAR ADENOMA								X					X	X			X			X	X		X		12
HEPATOCELLULAR CARCINOMA																X	X								10
MIXED HEPATO/CHOLANGIO CARCINOMA		X														X									1
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
GALLBLADDER & COMMON BILE DUCT	+	+	N	+	+	+	N	+	+	+	N	+	+	+	N	N	+	+	N	+	+	+	+	N	+	50*
PANCREAS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	-	+	46
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	49
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+	48
SQUAMOUS CELL CARCINOMA				X																					1
SMALL INTESTINE	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	-	+	45
LARGE INTESTINE	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	47
URINARY SYSTEM																									
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ENDOCRINE SYSTEM																									
PITUITARY	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	46
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
FOLLICULAR-CELL ADENOMA																									1
PARATHYROID	+	-	+	+	+	-	-	+	+	-	-	+	+	+	-	-	+	+	+	+	+	+	+	+	35
REPRODUCTIVE SYSTEM																									
MAMMARY GLAND	N	N	N	N	+	N	N	N	N	N	N	N	N	N	N	+	N	N	N	N	N	N	N	+	50*
TESTIS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
PROSTATE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
NERVOUS SYSTEM																									
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSE ORGANS																									
HARDERIAN GLAND	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50*
ADENOMA, NOS																									1
BODY CAVITIES																									
MEDIASTINUM	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50*
ALVEOLAR/BRONCHIOLAR CA, METASTAT		X																							1
ALL OTHER SYSTEMS																									
MULTIPLE ORGANS NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50*
SQUAMOUS CELL CARCINOMA, METASTAT				X																					1
FIBROSARCOMA																								X	1
HEMANGIOSARCOMA			X																						1
HEAD NOS																									
SARCOMA, NOS									X																1

* ANIMALS NECROPSIED

+: TISSUE EXAMINED MICROSCOPICALLY
-: REQUIRED TISSUE NOT EXAMINED MICROSCOPICALLY
X: TUMOR INCIDENCE
N: NECROPSY, NO AUTOLYSIS, NO MICROSCOPIC EXAMINATION

: NO TISSUE INFORMATION SUBMITTED
C: NECROPSY, NO HISTOLOGY DUE TO PROTOCOL
A: AUTOLYSIS
M: ANIMAL MISSING
B: NO NECROPSY PERFORMED

TABLE B4.

INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE 2-YEAR STUDY OF ALLYL ISOTHIOCYANATE

VEHICLE CONTROL

ANIMAL NUMBER	001	002	003	004	005	006	007	008	009	010	011	012	013	014	015	016	017	018	019	020	021	022	023	024	025
WEEKS ON STUDY	104	104	104	101	104	107	104	103	101	107	104	106	104	109	107	104	106	104	103	102	106	103	105	105	105
INTEGUMENTARY SYSTEM																									
SUBCUTANEOUS TISSUE HEMANGIOSARCOMA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+ X	+	+	+	+	+	+	+	+	+
RESPIRATORY SYSTEM																									
LUNGS AND BRONCHI ALVEOLAR/BRONCHIOLAR ADENOMA	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
TRACHEA	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
BONE MARROW	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPLEEN	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
THYMUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-
CIRCULATORY SYSTEM																									
HEART	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
LIVER HEPATOCELLULAR ADENOMA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GALLBLADDER & COMMON BILE DUCT	+	+	+	N	+	+	+	N	+	N	+	+	N	+	+	+	+	+	+	+	+	N	+	+	+
PANCREAS	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SMALL INTESTINE	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+
LARGE INTESTINE	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
URINARY SYSTEM																									
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY BLADDER	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
PITUITARY CARCINOMA,NOS ADENOMA, NOS ACIDOPHIL CARCINOMA	+ X	+	+ X	+	+	+	+ X	+	+	+	+	+	-	+	+	+	+ X	+	+	+	+	+	+	+	+
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
THYROID FOLLICULAR-CELL ADENOMA	+	+	+ X	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PARATHYROID	-	+	+	-	-	+	-	-	-	+	-	+	-	+	-	+	-	+	-	+	+	+	-	+	+
REPRODUCTIVE SYSTEM																									
MAMMARY GLAND ADENOMA, NOS ADENOCARCINOMA, NOS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+ X	+	+	+	+	+ X	+	+	+
UTERUS ENDOMETRIAL STROMAL POLYP HEMANGIOSARCOMA	+	+	+	+	+	+ X	+	+	+	+	+	+	+	+	+	+	+	+ X	+	+	+	+	+	+	+
OVARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
BRAIN ACIDOPHIL CARCINOMA, INVASIVE	+ X	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																									
HARDERIAN GLAND ADENOMA, NOS CYSTADENOMA, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
ALL OTHER SYSTEMS																									
MULTIPLE ORGANS NOS MALIGNANT LYMPHOMA, NOS MALIG.LYMPHOMA, LYMPHOCYTIC TYPE MALIG.LYMPHOMA, HISTIOCYTIC TYPE	N	N	N	N	N	N	N	N	N	N	N	N X	N	N	N	N	N	N	N	N	N	N X	N	N	N

+: TISSUE EXAMINED MICROSCOPICALLY
-: REQUIRED TISSUE NOT EXAMINED MICROSCOPICALLY
X: TUMOR INCIDENCE
N: NECROPSY, NO AUTOLYSIS, NO MICROSCOPIC EXAMINATION

: NO TISSUE INFORMATION SUBMITTED
C: NECROPSY, NO HISTOLOGY DUE TO PROTOCOL
A: AUTOLYSIS
M: ANIMAL MISSING
B: NO NECROPSY PERFORMED

TABLE B4. FEMALE MICE: TUMOR PATHOLOGY (CONTINUED) VEHICLE CONTROL

ANIMAL NUMBER	0 2 6	0 2 7	0 2 8	0 2 9	0 3 0	0 3 1	0 3 2	0 3 3	0 3 4	0 3 5	0 3 6	0 3 7	0 3 8	0 3 9	0 4 0	0 4 1	0 4 2	0 4 3	0 4 4	0 4 5	0 4 6	0 4 7	0 4 8	0 4 9	0 5 0	TOTAL TISSUES TUMORS
WEEKS ON STUDY	1 0 4	1 0 6	1 0 6	1 0 6	1 0 9	1 0 4	1 0 4	1 0 7	1 0 8	1 0 6	1 0 9	1 0 1	1 0 7	1 0 8	1 0 6	1 0 9	1 0 9	1 0 5	1 0 9	1 0 8	1 0 5	1 0 0	1 0 9	1 0 8	1 0 9	
INTEGUMENTARY SYSTEM																										
SUBCUTANEOUS TISSUE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50*
HEMANGIOSARCOMA																										1
RESPIRATORY SYSTEM																										
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+	47
ALVEOLAR/BRONCHIOLAR ADENOMA					X											X										2
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	47
HEMATOPOIETIC SYSTEM																										
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
SPLEEN	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
THYMUS	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	-	+	44
CIRCULATORY SYSTEM																										
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
DIGESTIVE SYSTEM																										
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEPATOCELLULAR ADENOMA																					X	X				2
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
GALLBLADDER & COMMON BILE DUCT	+	+	+	+	+	+	N	+	N	N	+	N	+	+	N	+	+	+	N	+	+	+	+	+	+	50*
PANCREAS	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	47
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
STOMACH	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	47
SMALL INTESTINE	+	+	+	+	+	-	+	+	-	-	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-	48
LARGE INTESTINE	+	+	+	+	+	-	+	+	-	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-	42
URINARY SYSTEM																										
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY BLADDER	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	47
ENDOCRINE SYSTEM																										
PITUITARY	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	47
CARCINOMA,NOS		X																								3
ADENOMA, NOS	X		X																							3
ACIDOPHIL CARCINOMA																										1
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
THYROID	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
FOLLICULAR-CELL ADENOMA																										1
PARATHYROID	+	+	-	+	+	+	+	-	+	-	-	-	+	+	+	+	-	+	+	+	+	-	+	+	-	30
REPRODUCTIVE SYSTEM																										
MAMMARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50*
ADENOMA, NOS																										1
ADENOCARCINOMA, NOS																										1
UTERUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ENDOMETRIAL STROMAL POLYP					X																					2
HEMANGIOSARCOMA																										1
OVARY	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
NERVOUS SYSTEM																										
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ACIDOPHIL CARCINOMA, INVASIVE																										1
SPECIAL SENSE ORGANS																										
HARDERIAN GLAND	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50*
ADENOMA, NOS																			X							1
CYSTADENOMA, NOS							X																			1
ALL OTHER SYSTEMS																										
MULTIPLE ORGANS NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50*
MALIGNANT LYMPHOMA, NOS																										1
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE																						X	X			3
MALIG.LYMPHOMA, HISTIOCYTIC TYPE																			X							1

* ANIMALS NECROPSIED

+: TISSUE EXAMINED MICROSCOPICALLY
-: REQUIRED TISSUE NOT EXAMINED MICROSCOPICALLY
X: TUMOR INCIDENCE
N: NECROPSY, NO AUTOLYSIS, NO MICROSCOPIC EXAMINATION

: NO TISSUE INFORMATION SUBMITTED
C: NECROPSY, NO HISTOLOGY DUE TO PROTOCOL
A: AUTOLYSIS
M: ANIMAL MISSING
B: NO NECROPSY PERFORMED

TABLE B4.

INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE 2-YEAR STUDY OF ALLYL ISOTHIOCYANATE

LOW DOSE

ANIMAL NUMBER	001	002	003	004	005	006	007	008	009	010	011	012	013	014	015	016	017	018	019	020	021	022	023	024	025
WEEKS ON STUDY	091	095	057	053	028	095	067	085	033	051	095	085	065	097	095	085	095	095	074	076	051	015	051	051	051
INTEGUMENTARY SYSTEM																									
SKIN HEMANGIOMA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+
SUBCUTANEOUS TISSUE MALIGNANT MELANOMA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+
RESPIRATORY SYSTEM																									
LUNGS AND BRONCHI ALVEOLAR/BRONCHIOLAR ADENOMA ALVEOLAR/BRONCHIOLAR CARCINOMA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+
TRACHEA	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+
LYMPH NODES MALIGNANT LYMPHOMA, MIXED TYPE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+
THYMUS	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	A	+	+
CIRCULATORY SYSTEM																									
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+
LIVER HEPATOCELLULAR ADENOMA HEPATOCELLULAR CARCINOMA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+
GALLBLADDER & COMMON BILE DUCT	+	+	N	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	N	+	N	+	+
PANCREAS	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	A	+	+
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
STOMACH SQUAMOUS CELL PAPILLOMA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+
SMALL INTESTINE	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	A	+	+
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	A	+	+
URINARY SYSTEM																									
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	A	+	+
ENDOCRINE SYSTEM																									
PITUITARY CARCINOMA,NOS ADENOMA, NOS	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+
THYROID FOLLICULAR-CELL ADENOMA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	-	+
PARATHYROID	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	A	-	+
PANCREATIC ISLETS ISLET-CELL ADENOMA	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	A	+	+
REPRODUCTIVE SYSTEM																									
MAMMARY GLAND ADENOCARCINOMA, NOS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+
UTERUS ADENOCARCINOMA, NOS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+
OVARY HEMANGIOSARCOMA	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	A	+	+
NERVOUS SYSTEM																									
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																									
HARDERIAN GLAND ADENOMA, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
BODY CAVITIES																									
MESENTERY HEMANGIOMA	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
ALL OTHER SYSTEMS																									
MULTIPLE ORGANS NOS MALIGNANT LYMPHOMA, NOS MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

+: TISSUE EXAMINED MICROSCOPICALLY
-: REQUIRED TISSUE NOT EXAMINED MICROSCOPICALLY
X: TUMOR INCIDENCE
N: NECROPSY, NO AUTOLYSIS, NO MICROSCOPIC EXAMINATION

: NO TISSUE INFORMATION SUBMITTED
C: NECROPSY, NO HISTOLOGY DUE TO PROTOCOL
A: AUTOLYSIS
M: ANIMAL MISSING
B: NO NECROPSY PERFORMED

TABLE B4. FEMALE MICE: TUMOR PATHOLOGY (CONTINUED) LOW DOSE

ANIMAL NUMBER	026	027	028	029	031	032	033	034	035	036	037	038	039	040	041	042	043	044	045	046	047	048	049	050	TOTAL TISSUES TUMORS
WEEKS ON STUDY	095	089	081	095	085	091	096	096	094	086	084	092	093	096	096	065	091	094	088	081	079	092	082	093	
INTEGUMENTARY SYSTEM																									
SKIN	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50N
HEMANGIOMA																						X			1
SUBCUTANEOUS TISSUE	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50N
MALIGNANT MELANOMA																									1
RESPIRATORY SYSTEM																									
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ALVEOLAR/BRONCHIOLAR ADENOMA																						X			1
ALVEOLAR/BRONCHIOLAR CARCINOMA																X						X			2
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	47
HEMATOPOIETIC SYSTEM																									
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	49
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	48
LYMPH NODES	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	47
MALIGNANT LYMPHOMA, MIXED TYPE																									1
THYMUS	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	45
CIRCULATORY SYSTEM																									
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																									
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
HEPATOCELLULAR ADENOMA																									1
HEPATOCELLULAR CARCINOMA													X												2
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
GALLBLADDER & COMMON BILE DUCT	+	N	+	+	+	+	+	+	+	+	N	N	+	+	+	+	+	+	N	+	N	+	+	+	5 8N
PANCREAS	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+	45
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
STOMACH	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+	47
SQUAMOUS CELL PAPILLOMA																									1
SMALL INTESTINE	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-	+	+	+	+	+	45
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-	+	+	+	+	+	45
URINARY SYSTEM																									
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	48
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	47
ENDOCRINE SYSTEM																									
PITUITARY	+	-	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	45
CARCINOMA,NOS																									3
ADENOMA, NOS																									3
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	47
THYROID	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	47
FOLLICULAR-CELL ADENOMA															X										1
PARATHYROID	+	+	+	+	+	+	-	-	+	-	+	-	+	+	+	+	+	+	+	+	-	+	+	+	48
PANCREATIC ISLETS	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+	45
ISLET-CELL ADENOMA																									1
REPRODUCTIVE SYSTEM																									
MAMMARY GLAND	+	+	+	+	+	+	+	+	+	+	N	+	+	N	+	+	K	+	+	+	+	+	+	+	50N
ADENOCARCINOMA, NOS																									1
UTERUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	47
ADENOCARCINOMA, NOS																						X			1
OVARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	44
HEMANGIOSARCOMA			X																						1
NERVOUS SYSTEM																									
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSE ORGANS																									
HARDERIAN GLAND	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50N
ADENOMA, NOS	X																								1
BODY CAVITIES																									
MESENTERY	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50N
HEMANGIOMA					X																				1
ALL OTHER SYSTEMS																									
MULTIPLE ORGANS NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50N
MALIGNANT LYMPHOMA, NOS																X									1
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE								X																	2

N: ANIMALS NECROPSIED

+: TISSUE EXAMINED MICROSCOPICALLY
-: REQUIRED TISSUE NOT EXAMINED MICROSCOPICALLY
X: TUMOR INCIDENCE
N: NECROPSY, NO AUTOLYSIS, NO MICROSCOPIC EXAMINATION

: NO TISSUE INFORMATION SUBMITTED
C: NECROPSY, NO HISTOLOGY DUE TO PROTOCOL
A: AUTOLYSIS
M: ANIMAL MISSING
D: NO NECROPSY PERFORMED

TABLE B4.

INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE 2-YEAR STUDY OF ALLYL ISOTHIOCYANATE

HIGH DOSE

ANIMAL NUMBER	001	002	003	004	005	006	007	008	009	010	011	012	013	014	015	016	017	018	019	020	021	022	023	024	025
WEEKS ON STUDY	065	095	104	104	124	075	087	078	078	052	080	090	041	051	093	096	091	104	078	081	015	082	084	078	104
INTEGUMENTARY SYSTEM																									
SUBCUTANEOUS TISSUE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
FIBROUS HISTIOCYTOMA, MALIGNANT																									
LYMPHANGIOMA																	X								
RESPIRATORY SYSTEM																									
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SQUAMOUS CELL CARCINOMA, METASTAT																									
ALVEOLAR/BRONCHIOLAR CARCINOMA						X							X			X									
OSTEOSARCOMA, METASTATIC																			X						
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMANGIOSARCOMA																					X				
MALIGNANT LYMPHOMA, MIXED TYPE																					X				
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
THYMUS	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+
CIRCULATORY SYSTEM																									
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEPATOCELLULAR CARCINOMA																									
KUPFFER-CELL SARCOMA																									
UNDIFFERENTIATED LEUKEMIA																						X			
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GALLBLADDER & COMMON BILE DUCT	+	+	+	+	+	+	+	M	+	+	M	+	+	M	+	+	+	+	+	M	+	+	+	+	+
PANCREAS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SMALL INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY BLADDER	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
PITUITARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ADENOMA, NOS													X		X										X
ADRENAL	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
THYROID	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
FOLLICULAR-CELL ADENOMA																									
FOLLICULAR-CELL CARCINOMA										X			X												
PARATHYROID	-	-	+	+	-	-	-	+	+	+	-	+	+	-	-	+	+	+	+	+	+	+	+	+	+
REPRODUCTIVE SYSTEM																									
MAMMARY GLAND	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ADENOCARCINOMA, NOS															X										
UTERUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SQUAMOUS CELL CARCINOMA																									
OVARY	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
TERATOMA, NOS														X											
NERVOUS SYSTEM																									
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
MUSCULOSKELETAL SYSTEM																									
BONE	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
OSTEOSARCOMA																			X						
ALL OTHER SYSTEMS																									
MULTIPLE ORGANS NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
FIBROUS HISTIOCYTOMA, MALIGNANT																									
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE																									
MALIG.LYMPHOMA, HISTIOCYTIC TYPE															X			X							
LYMPHOCYTIC LEUKEMIA						X																			

+: TISSUE EXAMINED MICROSCOPICALLY
-: REQUIRED TISSUE NOT EXAMINED MICROSCOPICALLY
X: TUMOR INCIDENCE
N: NECROPSY, NO AUTOLYSIS, NO MICROSCOPIC EXAMINATION

: NO TISSUE INFORMATION SUBMITTED
C: NECROPSY, NO HISTOLOGY DUE TO PROTOCOL
A: AUTOLYSIS
M: ANIMAL MISSING
B: NO NECROPSY PERFORMED

TABLE B4. FEMALE MICE: TUMOR PATHOLOGY (CONTINUED) HIGH DOSE

ANIMAL NUMBER / WEEKS ON STUDY	0 2 4 1 8 1	0 2 7 1 9 1	0 2 8 1 0 4	0 2 9 1 0 6	0 3 1 1 8 3	0 3 3 1 8 7	0 3 3 1 9 3	0 3 3 2 0 7	0 3 3 2 1 4	0 3 3 2 1 5	0 3 3 2 1 6	0 3 3 2 1 7	0 3 3 2 1 8	0 3 3 2 2 1	0 3 4 0 0 2	0 4 1 1 2 4	0 4 1 1 2 6	0 4 1 2 1 1	0 4 1 2 1 4	0 4 1 2 1 6	0 4 1 2 1 7	0 4 1 2 2 1	0 4 1 2 2 3	0 4 1 2 2 5	0 4 1 2 2 7	0 4 1 2 2 9	0 4 1 2 3 1	0 4 1 2 3 3	0 4 1 2 3 5	TOTAL TISSUES TUMORS
INTEGUMENTARY SYSTEM																														
SUBCUTANEOUS TISSUE	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
FIBROUS HISTIOCYTOMA, MALIGNANT														X																1
LYMPHANGIOMA																														1
RESPIRATORY SYSTEM																														
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
SQUAMOUS CELL CARCINOMA, METASTAT																									X					1
ALVEOLAR/BRONCHIOLAR CARCINOMA																														3
OSTEOSARCOMA, METASTATIC																														1
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	-	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
HEMATOPOIETIC SYSTEM																														
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
HEMANGIOSARCOMA																														1
MALIGNANT LYMPHOMA, MIXED TYPE																														1
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
THYMUS	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	44
CIRCULATORY SYSTEM																														
HEART	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
DIGESTIVE SYSTEM																														
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
HEPATOCELLULAR CARCINOMA					X																	X								1
KUPFFER-CELL SARCOMA																														1
UNDIFFERENTIATED LEUKEMIA																														1
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
GALLBLADDER & COMMON BILE DUCT	+	+	+	+	+	+	+	M	+	+	+	M	A	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	49
PANCREAS	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
SMALL INTESTINE	+	+	+	+	+	+	+	+	+	+	+	-	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	-	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
URINARY SYSTEM																														
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
ENDOCRINE SYSTEM																														
PITUITARY	+	+	+	+	-	+	+	+	+	+	+	+	A	+	+	-	+	+	+	-	+	+	-	-	+	+	+	+	+	44
ADENOMA, NOS															X															4
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	A	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
FOLLICULAR-CELL ADENOMA																		X												1
FOLLICULAR-CELL CARCINOMA																														2
PARATHYROID	+	+	-	+	+	+	-	-	+	+	+	+	A	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	37
REPRODUCTIVE SYSTEM																														
MAMMARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ADENOCARCINOMA, NOS																														1
UTERUS	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
SQUAMOUS CELL CARCINOMA																									X					1
OVARY	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
TERATOMA, NOS																														1
NERVOUS SYSTEM																														
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
MUSCULOSKELETAL SYSTEM																														
BONE	M	M	M	M	M	M	M	M	M	M	M	M	A	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	49
OSTEOSARCOMA																														1
ALL OTHER SYSTEMS																														
MULTIPLE ORGANS NOS	M	M	M	M	M	M	M	M	M	M	M	M	A	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	49
FIBROUS HISTIOCYTOMA, MALIGNANT																				X										1
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE									X																					1
MALIG.LYMPHOMA, HISTIOCYTIC TYPE																														2
LYMPHOCYTIC LEUKEMIA																														1

* ANIMALS NECROPSIED

+: TISSUE EXAMINED MICROSCOPICALLY
-: REQUIRED TISSUE NOT EXAMINED MICROSCOPICALLY
X: TUMOR INCIDENCE
M: NECROPSY, NO AUTOLYSIS, NO MICROSCOPIC EXAMINATION

: NO TISSUE INFORMATION SUBMITTED
C: NECROPSY, NO HISTOLOGY DUE TO PROTOCOL
A: AUTOLYSIS
M: ANIMAL MISSING
B: NO NECROPSY PERFORMED

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APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS ADMINISTERED ALLYL ISOTHIOCYANATE BY GAVAGE

TABLE C1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS ADMINISTERED ALLYL ISOTHIOCYANATE IN CORN OIL BY GAVAGE

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(50)
EPIDERMAL INCLUSION CYST	3 (6%)	1 (2%)	3 (6%)
*SUBCUT TISSUE	(50)	(50)	(50)
HEMATOMA, NOS		1 (2%)	
GRANULOMA, FOREIGN BODY	1 (2%)		
FIBROSIS	1 (2%)		
RESPIRATORY SYSTEM			
#LUNG	(49)	(49)	(48)
EDEMA, NOS	1 (2%)		
PNEUMONIA, ASPIRATION	1 (2%)		4 (8%)
INFLAMMATION, SUPPURATIVE	2 (4%)		
BRONCHOPNEUMONIA, CHRONIC			
CHOLESTEROL DEPOSIT			1 (2%)
HYPERPLASIA, ADENOMATOUS			1 (2%)
HYPERPLASIA, ALVEOLAR EPITHELIUM		3 (6%)	1 (2%)
METAPLASIA, OSSEOUS			1 (2%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(48)	(49)	(50)
HYPERPLASIA, NOS	2 (4%)		
MYELOFIBROSIS	1 (2%)		
#SPLEEN	(50)	(49)	(50)
CONGESTION, NOS	2 (4%)		
FIBROSIS, MULTIFOCAL	1 (2%)		
METAMORPHOSIS FATTY	1 (2%)		
HEMOSIDEROSIS	20 (40%)	20 (41%)	7 (14%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANGIECTASIS	1 (2%)		
HYPERPLASIA, LYMPHOID	2 (4%)		
HEMATOPOIESIS	1 (2%)	2 (4%)	
#LYMPH NODE	(50)	(50)	(50)
HYPERPLASIA, NOS	1 (2%)		
#MANDIBULAR L. NODE	(50)	(50)	(50)
HYPERPLASIA, PLASMA CELL		1 (2%)	
#MESENTERIC L. NODE	(50)	(50)	(50)
HEMORRHAGE, CHRONIC			
INFLAMMATION, GRANULOMATOUS			
ANGIECTASIS	1 (2%)		
#INGUINAL LYMPH NODE	(50)	(50)	(50)
HYPERPLASIA, DIFFUSE			
#PANCREAS	(50)	(50)	(49)
HYPERPLASIA, LYMPHOID	1 (2%)		
CIRCULATORY SYSTEM			
#HEART	(50)	(50)	(50)
MINERALIZATION	1 (2%)		
INFLAMMATION, CHRONIC			
INFLAMMATION, CHRONIC FOCAL	1 (2%)	1 (2%)	1 (2%)
FIBROSIS, FOCAL	23 (46%)	23 (46%)	19 (38%)
#MYOCARDIUM	(50)	(50)	(50)
INFLAMMATION, CHRONIC		2 (4%)	5 (10%)
INFLAMMATION, CHRONIC FOCAL			1 (2%)
*DESCENDING THORACIC	(50)	(50)	(50)
ARTERIOSCLEROSIS, NOS	1 (2%)		
*MESENTERIC ARTERY	(50)	(50)	(50)
INFLAMMATION, CHRONIC			1 (2%)
#PANCREAS	(50)	(50)	(49)
PERIARTERITIS			
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(49)	(50)	(50)
FIBROSIS, FOCAL		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ATROPHY, FOCAL		1 (2%)	
#LIVER	(50)	(50)	(50)
CONGESTION, ACUTE			1 (2%)
INFLAMMATION, GRANULOMATOUS			1 (2%)
NECROSIS, ZONAL	1 (2%)		
CYTOPLASMIC VACUOLIZATION	2 (4%)		
CYTOLOGIC ALTERATION, NOS	3 (6%)	4 (8%)	1 (2%)
ANGIECTASIS			2 (4%)
#LIVER/CENTRILOBULAR	(50)	(50)	(50)
CYTOPLASMIC VACUOLIZATION		1 (2%)	
#BILE DUCT	(50)	(50)	(50)
HYPERPLASIA, NOS	11 (22%)	32 (64%)	10 (20%)
HYPERPLASIA, FOCAL	14 (28%)	1 (2%)	1 (2%)
#PANCREAS	(50)	(50)	(49)
CYST, NOS			1 (2%)
ATROPHY, FOCAL	4 (8%)	5 (10%)	1 (2%)
#PANCREATIC ACINUS	(50)	(50)	(49)
ATROPHY, NOS	1 (2%)		
#GASTRIC SUBMUCOSA	(49)	(50)	(49)
FIBROSIS		1 (2%)	
#COLON	(48)	(49)	(49)
PARASITISM		1 (2%)	1 (2%)
URINARY SYSTEM			
#KIDNEY	(50)	(50)	(50)
INFLAMMATION, CHRONIC	40 (80%)	23 (46%)	20 (40%)
NEPHROSIS, NOS		1 (2%)	1 (2%)
PIGMENTATION, NOS		1 (2%)	
#KIDNEY/TUBULE	(50)	(50)	(50)
DEGENERATION, HYALINE	1 (2%)		
#URINARY BLADDER	(49)	(49)	(49)
INFLAMMATION, HEMORRHAGIC			
HYPERPLASIA, NODULAR			1 (2%)
HYPERPLASIA, EPITHELIAL		1 (2%)	6 (12%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#PITUITARY	(47)	(49)	(49)
ANGIECTASIS		1 (2%)	1 (2%)
#ADRENAL	(50)	(50)	(50)
CYST, NOS		1 (2%)	
CYTOPLASMIC VACUOLIZATION		1 (2%)	
#ADRENAL CORTEX	(50)	(50)	(50)
CYTOPLASMIC VACUOLIZATION		2 (4%)	
ANGIECTASIS	1 (2%)		
#ADRENAL MEDULLA	(50)	(50)	(50)
NECROSIS, NOS		1 (2%)	
HYPERPLASIA, FOCAL	2 (4%)		
#THYROID	(48)	(50)	(50)
CYSTIC FOLLICLES	7 (15%)	1 (2%)	1 (2%)
HYPERPLASIA, C-CELL		3 (6%)	
#PARATHYROID	(42)	(50)	(45)
HYPERPLASIA, NOS			
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
CYSTIC DUCTS	13 (26%)	15 (30%)	6 (12%)
HYPERPLASIA, NOS			1 (2%)
ADENOSIS	3 (6%)		
*PENIS	(50)	(50)	(50)
PROLAPSE	1 (2%)		
*PREPUTIAL GLAND	(50)	(50)	(50)
CYST, NOS		1 (2%)	2 (4%)
CYSTIC DUCTS	6 (12%)	3 (6%)	1 (2%)
INFLAMMATION, ACUTE			
INFLAMMATION, ACUTE SUPPURATIVE	1 (2%)		
INFLAMMATION ACUTE AND CHRONIC			1 (2%)
INFLAMMATION, CHRONIC SUPPURATIV		1 (2%)	
HYPERPLASIA, NOS			
HYPERPLASIA, CYSTIC			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
#PROSTATE	(49)	(49)	(49)
INFLAMMATION, SUPPURATIVE		1 (2%)	
INFLAMMATION, ACUTE SUPPURATIVE	10 (20%)	4 (8%)	1 (2%)
INFLAMMATION, CHRONIC FOCAL			
#PROSTATIC GLAND	(49)	(49)	(49)
ABSCESS, CHRONIC		1 (2%)	
*SEMINAL VESICLE	(50)	(50)	(50)
DILATATION, NOS		1 (2%)	1 (2%)
CYST, NOS			
INFLAMMATION, ACUTE FOCAL			
GRANULOMA, SPERMATIC			
#TESTIS	(50)	(50)	(49)
ATROPHY, NOS		1 (2%)	
NERVOUS SYSTEM			
#BRAIN/MENINGES	(50)	(49)	(50)
INFLAMMATION, CHRONIC FOCAL			1 (2%)
#HYPOTHALAMUS	(50)	(49)	(50)
HEMORRHAGE			
SPECIAL SENSE ORGANS			
*EYE	(50)	(50)	(50)
RETINOPATHY	9 (18%)	6 (12%)	39 (78%)
CATARACT	7 (14%)	6 (12%)	13 (26%)
MUSCULOSKELETAL SYSTEM			
*SKELETAL MUSCLE	(50)	(50)	(50)
DEGENERATION, NOS	1 (2%)		
BODY CAVITIES			
*MESENTERY	(50)	(50)	(50)
INFLAMMATION ACUTE AND CHRONIC		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
NECROSIS, FAT	14 (28%)	9 (18%)	8 (16%)
ANGIECTASIS			1 (2%)

ALL OTHER SYSTEMS

NONE

SPECIAL MORPHOLOGY SUMMARY

NONE

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS ADMINISTERED ALLYL ISOTHIOCYANATE IN CORN OIL BY GAVAGE

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(50)
INFLAMMATION, SUPPURATIVE			
INFLAMMATION, ACUTE FOCAL		1 (2%)	
INFLAMMATION ACUTE AND CHRONIC		1 (2%)	
INFLAMMATION, CHRONIC		1 (2%)	
*SUBCUT TISSUE	(50)	(50)	(50)
EPIDERMAL INCLUSION CYST	1 (2%)		
INFLAMMATION, CHRONIC FOCAL	1 (2%)		
RESPIRATORY SYSTEM			
#LUNG	(50)	(50)	(50)
EPIDERMAL INCLUSION CYST			1 (2%)
CONGESTION, NOS		1 (2%)	
HEMORRHAGE		2 (4%)	
PROTEINOSIS, ALVEOLAR		1 (2%)	
CHOLESTEROL DEPOSIT	1 (2%)		
HYPERPLASIA, ALVEOLAR EPITHELIUM			
HEMATOPOIETIC SYSTEM			
#SPLEEN	(50)	(50)	(50)
INFLAMMATION, CHRONIC			1 (2%)
FIBROSIS, FOCAL	1 (2%)		
HEMOSIDEROSIS	30 (60%)	30 (60%)	27 (54%)
ANGIECTASIS			1 (2%)
HEMATOPOIESIS	1 (2%)	1 (2%)	
#MEDIASTINAL L.NODE	(50)	(50)	(50)
HEMOSIDEROSIS	1 (2%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
#PANCREATIC L.NODE	(50)	(50)	(50)
ANGIECTASIS			
#PEYER'S PATCH	(49)	(48)	(48)
HYPERPLASIA, LYMPHOID			1 (2%)
CIRCULATORY SYSTEM			
#HEART	(50)	(50)	(50)
INFLAMMATION, CHRONIC FOCAL	1 (2%)	1 (2%)	1 (2%)
FIBROSIS, FOCAL	10 (20%)	8 (16%)	8 (16%)
#MYOCARDIUM	(50)	(50)	(50)
INFLAMMATION, CHRONIC		2 (4%)	
*MESENTERIC ARTERY	(50)	(50)	(50)
HEMORRHAGE		1 (2%)	
*MESENTERY	(50)	(50)	(50)
PERIARTERITIS		1 (2%)	
#KIDNEY/GLOMERULUS	(50)	(50)	(50)
EMBOLISM, NOS		1 (2%)	
#ADRENAL	(50)	(50)	(50)
EMBOLISM, NOS		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(50)	(50)	(50)
NECROSIS, FOCAL		1 (2%)	
NECROSIS, ZONAL		1 (2%)	
CYTOPLASMIC VACUOLIZATION			
CYTOLOGIC ALTERATION, NOS	3 (6%)	2 (4%)	3 (6%)
HYPERPLASIA, NOS			1 (2%)
#BILE DUCT	(50)	(50)	(50)
HYPERPLASIA, NOS	8 (16%)	21 (42%)	23 (46%)
HYPERPLASIA, FOCAL	12 (24%)	4 (8%)	1 (2%)
#PANCREAS	(49)	(49)	(50)
INFLAMMATION, CHRONIC	1 (2%)		1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, CHRONIC FOCAL		1 (2%)	
FIBROSIS, FOCAL	1 (2%)		
ATROPHY, NOS		1 (2%)	1 (2%)
ATROPHY, FOCAL	3 (6%)	3 (6%)	1 (2%)
#COLON	(49)	(47)	(49)
PARASITISM	1 (2%)		1 (2%)
URINARY SYSTEM			
#KIDNEY	(50)	(50)	(50)
INFLAMMATION, CHRONIC	1 (2%)	2 (4%)	
FIBROSIS, FOCAL	1 (2%)	1 (2%)	
NEPHROSIS, NOS			1 (2%)
PIGMENTATION, NOS		1 (2%)	
#URINARY BLADDER	(49)	(49)	(50)
HYPERPLASIA, EPITHELIAL			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(49)	(50)	(50)
CYST, NOS	1 (2%)		
HEMOSIDEROSIS	1 (2%)		
ANGIECTASIS	2 (4%)	3 (6%)	1 (2%)
#ADRENAL	(50)	(50)	(50)
CYTOPLASMIC VACUOLIZATION			2 (4%)
ANGIECTASIS		1 (2%)	
#ADRENAL CORTEX	(50)	(50)	(50)
CYTOPLASMIC VACUOLIZATION	5 (10%)	6 (12%)	3 (6%)
ANGIECTASIS			1 (2%)
#THYROID	(50)	(48)	(50)
ULTIMOBRANCHIAL CYST			1 (2%)
CYSTIC FOLLICLES	2 (4%)	1 (2%)	
FOLLICULAR CYST, NOS			
ATROPHY, CYSTIC		1 (2%)	
HYPERPLASIA, EPITHELIAL			1 (2%)
HYPERPLASIA, C-CELL		1 (2%)	1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
CYSTIC DUCTS	30 (60%)	30 (60%)	36 (72%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, NOS			3 (6%)
HYPERPLASIA, CYSTIC			5 (10%)
ADENOSIS	5 (10%)	9 (18%)	
*PREPUTIAL GLAND	(50)	(50)	(50)
CYSTIC DUCTS	1 (2%)	6 (12%)	2 (4%)
INFLAMMATION, ACUTE SUPPURATIVE		3 (6%)	1 (2%)
HYPERPLASIA, NOS	1 (2%)	1 (2%)	1 (2%)
*CLITORAL GLAND	(50)	(50)	(50)
CYST, NOS		1 (2%)	
CYSTIC DUCTS		1 (2%)	1 (2%)
INFLAMMATION, ACUTE SUPPURATIVE			
#UTERUS	(50)	(49)	(50)
HEMATOMETRA	1 (2%)	1 (2%)	1 (2%)
HYPERPLASIA, EPITHELIAL			1 (2%)
ANGIECTASIS			
#UTERUS/ENDOMETRIUM	(50)	(49)	(50)
EDEMA, NOS		1 (2%)	
HEMATOMETRA		2 (4%)	
INFLAMMATION, NOS		1 (2%)	
INFLAMMATION, ACUTE SUPPURATIVE	1 (2%)		3 (6%)
HYPERPLASIA, NOS	9 (18%)	5 (10%)	2 (4%)
HYPERPLASIA, CYSTIC			
#ENDOMETRIAL GLAND	(50)	(49)	(50)
HYPERPLASIA, CYSTIC		1 (2%)	
#OVARY	(50)	(50)	(50)
CYST, NOS	3 (6%)		1 (2%)
FOLLICULAR CYST, NOS		1 (2%)	
NERVOUS SYSTEM			
#CEREBRAL VENTRICLE	(50)	(50)	(50)
HYDROCEPHALUS, NOS			1 (2%)
SPECIAL SENSE ORGANS			
*EYE	(50)	(50)	(50)
RETINOPATHY	4 (8%)	35 (70%)	11 (22%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
CATARACT	2 (4%)	33 (66%)	9 (18%)
*EYE/RETINA	(50)	(50)	(50)
DEGENERATION, NOS		1 (2%)	
*EYELID	(50)	(50)	(50)
INFLAMMATION, CHRONIC FOCAL		1 (2%)	
MUSCULOSKELETAL SYSTEM			
*STERNUM	(50)	(50)	(50)
CYST, NOS			
BODY CAVITIES			
*MEDIASTINAL PLEURA	(50)	(50)	(50)
HEMORRHAGE		1 (2%)	
*MESENTERY	(50)	(50)	(50)
MINERALIZATION		1 (2%)	
HEMORRHAGE			
FIBROSIS, FOCAL		1 (2%)	
NECROSIS, FAT	8 (16%)	18 (36%)	13 (26%)
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	3	1	

@ NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE ADMINISTERED ALLYL ISOTHIOCYANATE BY GAVAGE

TABLE D1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE ADMINISTERED ALLYL ISOTHIOCYANATE IN CORN OIL BY GAVAGE

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(50)
ULCER, FOCAL			
INFLAMMATION, ACUTE/CHRONIC			
INFLAMMATION, CHRONIC		1 (2%)	
FIBROSIS			
FIBROSIS, FOCAL	1 (2%)		
*SUBCUT TISSUE	(50)	(50)	(50)
INFLAMMATION, SUPPURATIVE			1 (2%)
INFLAMMATION, CHRONIC SUPPURATIV			2 (4%)
INFLAMMATION, FOCAL GRANULOMATOU		1 (2%)	
INFLAMMATION, PYOGRANULOMATOUS	1 (2%)		
NECROSIS, FOCAL			
NECROSIS, FAT			3 (6%)
FOREIGN MATERIAL, NOS		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG/BRONCHIOLE	(50)	(50)	(50)
HYPERPLASIA, NOS	2 (4%)		1 (2%)
#LUNG	(50)	(50)	(50)
EDEMA, NOS			1 (2%)
HEMORRHAGE	2 (4%)		
BRONCHOPNEUMONIA, FOCAL	2 (4%)		
LYMPHOCYTIC INFLAMMATORY INFILTR			
INFLAMMATION, INTERSTITIAL	1 (2%)		1 (2%)
BRONCHOPNEUMONIA SUPPURATIVE		1 (2%)	
INFLAMMATION, ACUTE/CHRONIC	1 (2%)		
PNEUMONIA, CHRONIC MURINE	3 (6%)		1 (2%)
INFLAMMATION, CHRONIC FOCAL			1 (2%)
INFLAMMATION, GRANULOMATOUS	1 (2%)	2 (4%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, FOCAL GRANULOMATOU	3 (6%)		
REACTION, FOREIGN BODY	1 (2%)		
CHOLESTEROL DEPOSIT		2 (4%)	
HYPERPLASIA, ADENOMATOUS	8 (16%)	12 (24%)	15 (30%)
HYPERPLASIA, ALVEOLAR EPITHELIUM	3 (6%)	1 (2%)	2 (4%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
HYPERPLASIA, HEMATOPOIETIC	1 (2%)		
HYPERPLASIA, LYMPHOID	1 (2%)		
#SPLEEN	(49)	(48)	(50)
ANGIECTASIS		1 (2%)	
HYPERPLASIA, LYMPHOID	1 (2%)		
HEMATOPOIESIS	2 (4%)		1 (2%)
#MANDIBULAR L. NODE	(50)	(49)	(49)
HYPERPLASIA, LYMPHOID			1 (2%)
#MESENTERIC L. NODE	(50)	(49)	(49)
HEMORRHAGE	2 (4%)		
ANGIECTASIS	1 (2%)	1 (2%)	1 (2%)
HYPERPLASIA, LYMPHOID	2 (4%)	1 (2%)	2 (4%)
HEMATOPOIESIS			
#INGUINAL LYMPH NODE	(50)	(49)	(49)
HYPERPLASIA, LYMPHOID	2 (4%)		1 (2%)
#LUNG/BRONCHUS	(50)	(50)	(50)
HYPERPLASIA, LYMPHOID			
#PEYER'S PATCH	(45)	(42)	(45)
HYPERPLASIA, LYMPHOID	5 (11%)	4 (10%)	2 (4%)
#THYMUS	(41)	(48)	(46)
CYST, NOS	1 (2%)		
ATROPHY, NOS			1 (2%)
HYPERPLASIA, LYMPHOID		1 (2%)	
CIRCULATORY SYSTEM			
#ILIAC LYMPH NODE	(50)	(49)	(49)
LYMPHANGIECTASIS			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
#AURICULAR APPENDAGE	(50)	(50)	(50)
PERIARTERITIS			
#MYOCARDIUM	(50)	(50)	(50)
INFLAMMATION, NECROTIZING			1 (2%)
INFLAMMATION, ACUTE/CHRONIC			1 (2%)
INFLAMMATION, CHRONIC FOCAL	2 (4%)		
*BLOOD VESSEL	(50)	(50)	(50)
DEGENERATION PIGMENTARY	1 (2%)		
*AORTA	(50)	(50)	(50)
CALCIFICATION, FOCAL	1 (2%)		
#LIVER	(49)	(49)	(50)
THROMBOSIS, NOS	1 (2%)		
*MESENTERY	(50)	(50)	(50)
PERIARTERITIS			1 (2%)
#KIDNEY	(49)	(49)	(50)
PERIARTERITIS	1 (2%)		1 (2%)
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(50)	(49)	(50)
HEMORRHAGE	1 (2%)		
INFLAMMATION, GRANULOMATOUS	1 (2%)		
FIBROSIS, FOCAL			1 (2%)
CHOLESTEROL DEPOSIT	1 (2%)		
#LIVER	(49)	(49)	(50)
INFLAMMATION, ACUTE FIBRINOUS	1 (2%)		
INFLAMMATION, ACUTE/CHRONIC	1 (2%)		
INFLAMMATION, CHRONIC SUPPURATIV	1 (2%)		
NECROSIS, NOS			1 (2%)
NECROSIS, COAGULATIVE	1 (2%)	1 (2%)	1 (2%)
CYTOPLASMIC CHANGE, NOS			
CYTOPLASMIC VACUOLIZATION		4 (8%)	10 (20%)
FOCAL CELLULAR CHANGE	2 (4%)	1 (2%)	2 (4%)
HYPERPLASIA, FOCAL	1 (2%)		
#LIVER/CENTRILOBULAR	(49)	(49)	(50)
CYTOPLASMIC VACUOLIZATION	2 (4%)	4 (8%)	3 (6%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
*GALLBLADDER	(50)	(50)	(50)
HYPERPLASIA, NOS	1 (2%)		
#BILE DUCT	(49)	(49)	(50)
CYST, NOS		1 (2%)	
#ESOPHAGUS	(50)	(50)	(49)
INFLAMMATION, CHRONIC		1 (2%)	
INFLAMMATION, GRANULOMATOUS	3 (6%)	1 (2%)	1 (2%)
#GASTRIC MUCOSA	(49)	(48)	(48)
EPIDERMAL INCLUSION CYST	1 (2%)		
#ILEUM	(45)	(42)	(45)
DIVERTICULUM		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(49)	(49)	(50)
PYELONEPHRITIS, FOCAL	1 (2%)		
INFLAMMATION, INTERSTITIAL	1 (2%)		2 (4%)
PYELONEPHRITIS, ACUTE/CHRONIC			
NEPHROPATHY	3 (6%)		
DEGENERATION PIGMENTARY	1 (2%)		
NEPHROSIS, NOS		1 (2%)	
METAPLASIA, OSSEOUS			1 (2%)
#KIDNEY/PELVIS	(49)	(49)	(50)
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(46)	(46)	(46)
CYST, NOS	1 (2%)		
#ADRENAL	(47)	(49)	(50)
CYTOLOGIC ALTERATION, NOS			
#ADRENAL MEDULLA	(47)	(49)	(50)
HYPERPLASIA, NOS			1 (2%)
#THYROID	(50)	(45)	(50)
CYSTIC FOLLICLES		1 (2%)	1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
FOLLICULAR CYST, NOS	2 (4%)		
INFLAMMATION, SUPPURATIVE	1 (2%)		
REACTION, FOREIGN BODY	1 (2%)		
DEGENERATION, CYSTIC	1 (2%)		
HYPERPLASIA, FOLLICULAR-CELL			1 (2%)
#THYROID FOLLICLE	(50)	(45)	(50)
HYPERPLASIA, CYSTIC	1 (2%)		
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(50)	(50)	(50)
EPIDERMAL INCLUSION CYST		1 (2%)	
CYSTIC DUCTS	2 (4%)	2 (4%)	6 (12%)
INFLAMMATION, SUPPURATIVE			1 (2%)
ABSCESS, NOS		1 (2%)	
INFLAMMATION, CHRONIC			1 (2%)
INFLAMMATION, CHRONIC SUPPURATIV	1 (2%)	1 (2%)	1 (2%)
#PROSTATE	(50)	(50)	(50)
INFLAMMATION, SUPPURATIVE	1 (2%)		
HYPERPLASIA, EPITHELIAL			1 (2%)
#TESTIS	(50)	(50)	(50)
NECROSIS, FOCAL	1 (2%)		
ATROPHY, NOS	1 (2%)		
HYPERPLASIA, INTERSTITIAL CELL		1 (2%)	
*EPIDIDYMIS	(50)	(50)	(50)
ULCER, NOS		1 (2%)	
NERVOUS SYSTEM			
#BRAIN	(50)	(50)	(50)
CORPORA AMYLACEA		1 (2%)	
SPECIAL SENSE ORGANS			
*EYE	(50)	(50)	(50)
ATROPHY, NOS	1 (2%)		
*EYE/RETINA	(50)	(50)	(50)
DEGENERATION, NOS	1 (2%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
MUSCULOSKELETAL SYSTEM			
*INTERCOSTAL MUSCLE	(50)	(50)	(50)
INFLAMMATION, NECROTIZING		1 (2%)	
BODY CAVITIES			
*MEDIASTINUM	(50)	(50)	(50)
INFLAMMATION, GRANULOMATOUS	1 (2%)		
INFLAMMATION, FOCAL GRANULOMATOU	1 (2%)	1 (2%)	
*PERICARDIUM	(50)	(50)	(50)
EDEMA, NOS		1 (2%)	
REACTION, FOREIGN BODY		1 (2%)	
NECROSIS, FAT		1 (2%)	
*MESENTERY	(50)	(50)	(50)
HEMORRHAGIC CYST	1 (2%)		
STEATITIS	2 (4%)		2 (4%)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (2%)		
NECROSIS, FAT	2 (4%)	1 (2%)	3 (6%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(50)	(50)
ULCER, FOCAL			1 (2%)
INFLAMMATION, SUPPURATIVE	1 (2%)		1 (2%)
INFLAMMATION, GRANULOMATOUS		1 (2%)	
OMENTUM			
STEATITIS	1		
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		10	5
AUTO/NECROPSY/NO HISTO			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE ADMINISTERED ALLYL ISOTHIOCYANATE IN CORN OIL BY GAVAGE

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	49
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(49)
EPIDERMAL INCLUSION CYST			1 (2%)
INFLAMMATION, GRANULOMATOUS		1 (2%)	
*SUBCUT TISSUE	(50)	(50)	(49)
ABSCESS, NOS		1 (2%)	
INFLAMMATION, FOCAL GRANULOMATOU			1 (2%)
REACTION, FOREIGN BODY		1 (2%)	
INFLAMMATION, PYOGRANULOMATOUS		1 (2%)	
CHOLESTEROL DEPOSIT		1 (2%)	1 (2%)
RESPIRATORY SYSTEM			
#TRACHEA	(47)	(47)	(48)
PENETRATING WOUND		1 (2%)	
#LUNG/BRONCHIOLE	(47)	(49)	(49)
HYPERPLASIA, NOS		1 (2%)	
#LUNG	(47)	(49)	(49)
HEMORRHAGE		1 (2%)	1 (2%)
INFLAMMATION, INTERSTITIAL			2 (4%)
PNEUMONIA, ASPIRATION		1 (2%)	
INFLAMMATION, SUPPURATIVE	1 (2%)		1 (2%)
BRONCHOPNEUMONIA SUPPURATIVE			1 (2%)
PNEUMONIA, CHRONIC MURINE	4 (9%)		2 (4%)
INFLAMMATION, GRANULOMATOUS		1 (2%)	1 (2%)
INFLAMMATION, FOCAL GRANULOMATOU			2 (4%)
CHOLESTEROL DEPOSIT		1 (2%)	1 (2%)
HYPERPLASIA, ADENOMATOUS	3 (6%)	2 (4%)	3 (6%)
HYPERPLASIA, ALVEOLAR EPITHELIUM	1 (2%)	2 (4%)	1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
HEMATOPOIETIC SYSTEM			
#BRAIN/MENINGES	(50)	(50)	(49)
HYPERPLASIA, LYMPHOID	1 (2%)		
*MULTIPLE ORGANS	(50)	(50)	(49)
HYPERPLASIA, HEMATOPOIETIC	3 (6%)		
HYPERPLASIA, LYMPHOID	2 (4%)		1 (2%)
HEMATOPOIESIS		1 (2%)	
#BONE MARROW	(49)	(49)	(49)
HYPERPLASIA, NOS	2 (4%)	2 (4%)	1 (2%)
MYELOFIBROSIS		4 (8%)	
HYPERPLASIA, HEMATOPOIETIC		2 (4%)	1 (2%)
HYPERPLASIA, GRANULOCYTIC		1 (2%)	4 (8%)
HYPERPLASIA, RETICULUM CELL	1 (2%)		
#SPLEEN	(47)	(48)	(49)
HYPERPLASIA, NOS		1 (2%)	
HYPERPLASIA, HEMATOPOIETIC			1 (2%)
HYPERPLASIA, LYMPHOID	2 (4%)	4 (8%)	2 (4%)
HEMATOPOIESIS	3 (6%)	10 (21%)	5 (10%)
MYELOPOIESIS			
#SPLENIC CAPSULE	(47)	(48)	(49)
INFLAMMATION, CHRONIC FOCAL		1 (2%)	
#LYMPH NODE	(50)	(47)	(49)
HYPERPLASIA, NOS	1 (2%)		
#MANDIBULAR L. NODE	(50)	(47)	(49)
HYPERPLASIA, LYMPHOID		1 (2%)	
#CERVICAL LYMPH NODE	(50)	(47)	(49)
HYPERPLASIA, LYMPHOID			1 (2%)
#PANCREATIC L.NODE	(50)	(47)	(49)
HYPERPLASIA, NOS			1 (2%)
#MESENTERIC L. NODE	(50)	(47)	(49)
HEMORRHAGIC CYST		1 (2%)	
INFLAMMATION, GRANULOMATOUS		1 (2%)	
#RENAL LYMPH NODE	(50)	(47)	(49)
HYPERPLASIA, NOS	1 (2%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, LYMPHOID		3 (6%)	
#ILIAC LYMPH NODE	(50)	(47)	(49)
ANGIECTASIS			
#LUNG/BRONCHUS	(47)	(49)	(49)
HYPERPLASIA, LYMPHOID			
#LUNG	(47)	(49)	(49)
HYPERPLASIA, LYMPHOID		1 (2%)	1 (2%)
#LIVER	(50)	(49)	(49)
LEUKOCYTOSIS, NOS	1 (2%)		
HEMATOPOIESIS	1 (2%)	4 (8%)	1 (2%)
MYELOPOIESIS			
#PEYER'S PATCH	(40)	(44)	(47)
HYPERPLASIA, LYMPHOID	1 (3%)		
#KIDNEY	(50)	(48)	(49)
PLASMACYTOSIS		1 (2%)	
HYPERPLASIA, LYMPHOID			1 (2%)
#THYMUS	(44)	(45)	(44)
INFLAMMATION, CHRONIC		1 (2%)	
ATROPHY, NOS			1 (2%)
HYPERPLASIA, LYMPHOID		1 (2%)	
CIRCULATORY SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(49)
PERIARTERITIS		1 (2%)	
#ENDOCARDIUM	(49)	(50)	(49)
FIBROSIS, FOCAL			1 (2%)
*AORTA	(50)	(50)	(49)
INFLAMMATION, ACUTE/CHRONIC		1 (2%)	
*CORONARY ARTERY	(50)	(50)	(49)
INFLAMMATION, NECROTIZING			1 (2%)
HYPERTROPHY, FOCAL			1 (2%)
#PANCREAS	(47)	(45)	(49)
PERIARTERITIS			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
#OVARY	(49)	(44)	(48)
THROMBOSIS, NOS		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(50)	(49)	(49)
HEMORRHAGIC CYST	1 (2%)		
INFLAMMATION, NOS			1 (2%)
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (2%)	
INFLAMMATION, ACUTE SUPPURATIVE	1 (2%)		
INFLAMMATION, ACUTE/CHRONIC	1 (2%)	3 (6%)	2 (4%)
INFLAMMATION, GRANULOMATOUS	1 (2%)		
NECROSIS, FOCAL	1 (2%)		
NUCLEAR ENLARGEMENT	1 (2%)		
INCLUSION, NUCLEAR	1 (2%)		
CYTOPLASMIC CHANGE, NOS			
CYTOPLASMIC VACUOLIZATION		1 (2%)	1 (2%)
FOCAL CELLULAR CHANGE		1 (2%)	1 (2%)
HYPERPLASIA, FOCAL	1 (2%)		
*GALLBLADDER	(50)	(50)	(49)
INFLAMMATION, SUPPURATIVE		1 (2%)	
#PANCREAS	(47)	(45)	(49)
CYSTIC DUCTS		2 (4%)	
EDEMA, NOS			1 (2%)
INFLAMMATION, INTERSTITIAL		1 (2%)	
INFLAMMATION, SUPPURATIVE		1 (2%)	1 (2%)
INFLAMMATION, CHRONIC	1 (2%)		
NECROSIS, FAT		1 (2%)	
ATROPHY, NOS			1 (2%)
*OROPHARYNX	(50)	(50)	(49)
INFLAMMATION, ACUTE/CHRONIC			1 (2%)
#ESOPHAGUS	(49)	(50)	(49)
PENETRATING WOUND		1 (2%)	
INFLAMMATION ACUTE AND CHRONIC			1 (2%)
INFLAMMATION, CHRONIC			1 (2%)
INFLAMMATION, CHRONIC SUPPURATIV		1 (2%)	
INFLAMMATION, GRANULOMATOUS	5 (10%)		
#CARDIAC STOMACH	(47)	(47)	(49)
ULCER, NOS			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, BASAL CELL	1 (2%)		
#INTESTINAL VILLUS	(40)	(44)	(47)
CYTOPLASMIC VACUOLIZATION			1 (2%)
URINARY SYSTEM			
#KIDNEY	(50)	(48)	(49)
HYDRONEPHROSIS			1 (2%)
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (2%)	
INFLAMMATION, INTERSTITIAL		1 (2%)	
INFLAMMATION, CHRONIC	1 (2%)		
NEPHROPATHY	1 (2%)		1 (2%)
NECROSIS, MEDULLARY		1 (2%)	
HYPOPLASIA, NOS			1 (2%)
#KIDNEY/PELVIS	(50)	(48)	(49)
LYMPHOCYTIC INFLAMMATORY INFILTR			
INFLAMMATION, NECROTIZING	1 (2%)	1 (2%)	
#URINARY BLADDER	(47)	(47)	(47)
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(47)	(45)	(44)
HYPERPLASIA, NOS	3 (6%)		
HYPERPLASIA, FOCAL			3 (7%)
ANGIECTASIS	1 (2%)	2 (4%)	
#THYROID	(48)	(47)	(47)
CYSTIC FOLLICLES	1 (2%)		1 (2%)
FOLLICULAR CYST, NOS			2 (4%)
DEGENERATION, CYSTIC	2 (4%)		
HYPERPLASIA, FOLLICULAR-CELL	1 (2%)		
#THYROID FOLLICLE	(48)	(47)	(47)
MULTIPLE CYSTS		1 (2%)	
HYPERPLASIA, PAPILLARY			1 (2%)
HYPERPLASIA, CYSTIC	1 (2%)		1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(49)
CYSTIC DUCTS	4 (8%)	3 (6%)	2 (4%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
FIBROSIS	1 (2%)		
HYPERPLASIA, NOS		2 (4%)	
*MAMMARY LOBULE	(50)	(50)	(49)
HYPERPLASIA, NOS	1 (2%)		
#UTERUS	(50)	(47)	(49)
HYDROMETRA		1 (2%)	
CYST, NOS	1 (2%)		1 (2%)
INFLAMMATION, SUPPURATIVE			1 (2%)
PYOMETRA	2 (4%)	1 (2%)	1 (2%)
ENDOMETRIAL POLYP		1 (2%)	
ANGIECTASIS	1 (2%)		
#UTERUS/ENDOMETRIUM	(50)	(47)	(49)
CYST, NOS	5 (10%)	3 (6%)	4 (8%)
INFLAMMATION, SUPPURATIVE	1 (2%)	3 (6%)	3 (6%)
HYPERPLASIA, NOS	1 (2%)		
HYPERPLASIA, CYSTIC	5 (10%)	2 (4%)	8 (16%)
HYPERPLASIA, ADENOMATOUS			
ANGIECTASIS			1 (2%)
#ENDOMETRIAL GLAND	(50)	(47)	(49)
HYPERPLASIA, CYSTIC	18 (36%)	25 (53%)	14 (29%)
#OVARY	(49)	(44)	(48)
CYST, NOS	2 (4%)	1 (2%)	3 (6%)
CYSTIC FOLLICLES	1 (2%)		
FOLLICULAR CYST, NOS		1 (2%)	
HEMATOMA, NOS	1 (2%)		
INFLAMMATION, SUPPURATIVE			1 (2%)
ABSCESS, CHRONIC	4 (8%)	2 (5%)	3 (6%)
NERVOUS SYSTEM			
#BRAIN/MENINGES	(50)	(50)	(49)
INFLAMMATION, SUPPURATIVE	1 (2%)		
#BRAIN	(50)	(50)	(49)
INFLAMMATION, ACUTE/CHRONIC	1 (2%)		
CORPORA AMYLACEA			
#BRAIN/THALAMUS	(50)	(50)	(49)
PSAMMOMA BODIES	1 (2%)		1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
SPECIAL SENSE ORGANS			
*EYE	(50)	(50)	(49)
PHTHISIS BULBI		1 (2%)	
*MIDDLE EAR	(50)	(50)	(49)
INFLAMMATION, SUPPURATIVE	2 (4%)	1 (2%)	
INFLAMMATION, CHRONIC SUPPURATIV			1 (2%)
MUSCULOSKELETAL SYSTEM			
*BONE	(50)	(50)	(49)
FIBROUS DYSPLASIA	1 (2%)		
*CORTEX OF BONE	(50)	(50)	(49)
FIBROUS OSTEODYSTROPHY			3 (6%)
HYPERPLASIA, NOS		1 (2%)	
BODY CAVITIES			
*THORACIC CAVITY	(50)	(50)	(49)
INFLAMMATION, SUPPURATIVE			1 (2%)
REACTION, FOREIGN BODY			1 (2%)
*MEDIASTINUM	(50)	(50)	(49)
INFLAMMATION, GRANULOMATOUS	2 (4%)		1 (2%)
*PERITONEUM	(50)	(50)	(49)
INFLAMMATION, SUPPURATIVE	1 (2%)		1 (2%)
INFLAMMATION, FIBRINOUS	1 (2%)		
INFLAMMATION, ACUTE SUPPURATIVE	2 (4%)		
INFLAMMATION, CHRONIC			1 (2%)
INFLAMMATION, CHRONIC SUPPURATIV	1 (2%)		2 (4%)
ABSCESS, CHRONIC	1 (2%)		
NECROSIS, FAT			1 (2%)
*PLEURA	(50)	(50)	(49)
INFLAMMATION, SUPPURATIVE			1 (2%)
INFLAMMATION, ACUTE SUPPURATIVE	1 (2%)		
*MEDIASTINAL PLEURA	(50)	(50)	(49)
INFLAMMATION, CHRONIC SUPPURATIV		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
REACTION, FOREIGN BODY		1 (2%)	
*MESENTERY	(50)	(50)	(49)
STEATITIS		1 (2%)	1 (2%)
INFLAMMATION, NECROTIZING	1 (2%)		
INFLAMMATION, CHRONIC	1 (2%)		
INFLAMMATION, CHRONIC SUPPURATIV	2 (4%)		
NECROSIS, FAT	1 (2%)	3 (6%)	
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(50)	(49)
LYMPHOCYTIC INFLAMMATORY INFILTR			
INFLAMMATION, SUPPURATIVE	9 (18%)	5 (10%)	4 (8%)
INFLAMMATION, ACUTE FIBRINOUS			
HYPERPLASIA, NOS		1 (2%)	
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	3	3	2
AUTO/NECROPSY/HISTO PERF		1	
AUTOLYSIS/NO NECROPSY			1

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

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APPENDIX E

ANALYSIS OF ALLYL ISOTHIOCYANATE
LOT NO. 532251
(MIDWEST RESEARCH INSTITUTE)

A. ELEMENTAL ANALYSIS

Element	C	H	N	S
Theory	48.45	5.08	14.13	32.34
Determined	48.52	5.08	14.10	32.13
	48.56	5.13	14.18	32.27

B. BOILING POINT

Determined

151°C at 746.3 mm (visual, micro boiling point tube) 148° to 152°C (Dupont 900DTA)

Literature Values

152.05°C at 760 mm (Timmermans and Hennault-Roland, 1922)

C. DENSITY

Determined

d_{22}^{23}:1.016

Literature Value

d_4^{30}:1.00811 (variation 0.000103/°C) (Timmermans and Hennault-Roland, 1922)

D. REFRACTIVE INDEX

Determined

n_D^{20} 1.5315 ± 0.0002 (δ)

Literature Value

n_D^{17} 1.5336 (Timmermans and Hennault-Roland, 1922)

E. THIN-LAYER CHROMATOGRAPHY

Plates: Silica Gel 60 F254
Amount spotted: 100 and 300 μg

System 1: 95% Ethanol
R_f: 0.86
R_{st}: 1.13

System 2: Chloroform:1,4-Dioxane (95:5)
R_f: 0.55
R_{st}: 0.61

Ref. Standard: 1,1,3,3-Tetramethylthiourea
Visualization: Ultraviolet (254 nm), and I_2 vapor

F. VAPOR-PHASE CHROMATOGRAPHY

1. System 1

Instrument: Bendix 2500
Detector: Flame ionization
Column: Chromosorb 102, 1.8 m x 4 mm I.D.
Inlet temperature: 225°C
Detector temperature: 270°C
Oven temperature program: 2 min. at 150°C, then 150° to 200°C at 10°/min.
Results: Major peak and four impurities

Peak	Retention Time (min.)	Retention Time (Relative to Allyl Isothiocyanate)	Area (Relative to Allyl Isothiocyanate)
1	3.5	0.21	0.007
2	8.6	0.52	0.04
3	9.3	0.56	0.07
4	16.6	1.00	100
5	20.3	1.22	0.2

2. System 2

Instrument: Bendix 2500
Detector: Flame ionization
Column: 10% Carbowax 20 M, on 80/100 Chromosorb W (AW), 1.8 m x 4 mm I.D.
Inlet temperature: 225°C
Detector temperature: 270°C
Oven temperature program: 5 min. at 50°C, then 50° to 125°C at 10°C/min.
Results: Major peak and six impurities

Peak	Retention Time (min.)	Retention Time (Relative to Allyl Isothiocyanate)	Area (Relative to Allyl Isothiocyanate)
1	1.0	0.07	0.006
2	4.9	0.36	0.3
3	10.6	0.78	0.08
4	12.8	0.95	Shoulder 0.1%
5	13.5	1.00	100
6	15.2	1.13	0.5
7	16.0	1.19	0.04

G. SPECTRAL DATA

1. Infrared

Instrument: Beckman IR-12
Cell: Neat, sodium chloride plates
Results: See Figure 5

Consistent with literature spectrum (Sadtler Research Laboratories)

2. Ultraviolet/Visible

Instrument: Cary 118

Determined literature values (Sadtler Research Laboratories)

λ max (nm)	$\varepsilon \times 10^{-2}$
249	10.40 ± 0.01 (δ)

No absorbance between 350 and 800 nm (visible range) at a concentration of 1 mg/ml
Solvent: Hexane

λ max (nm)	$\varepsilon \times 10^{-2}$
247	8.30

(Calculated from graph of spectrum)

3. Nuclear Magnetic Resonance

Instrument: Varian HA-100
Solvent: Chloroform-d with internal tetramethylsilane
Assignments (See Figure 6)

Solvent: Dioxane

Identical to literature spectrum (Sadtler Research Laboratories)

(a) } d^2 δ 4.17 ppm
(b) } d
(c) m, δ 5.31 ppm
(d) m, δ 5.42 ppm
(e) t^4, δ 5.92 ppm
(f) d, δ 3.59 ppm (impurity, possibly thiocyanate)

J_{ae} = 4.7 Hz, J_{be} = 4.7 Hz, J_{ad} = 3.2 Hz, J_{cd} ▫ 1.5 Hz, J_{ce} ▫ 10 Hz, J_{de} = 17.5 Hz

Integration Ratios:

(a) } 1.82
(b) }
(c) } 2.00
(d) }
(e) 1.17
(f) 0.06

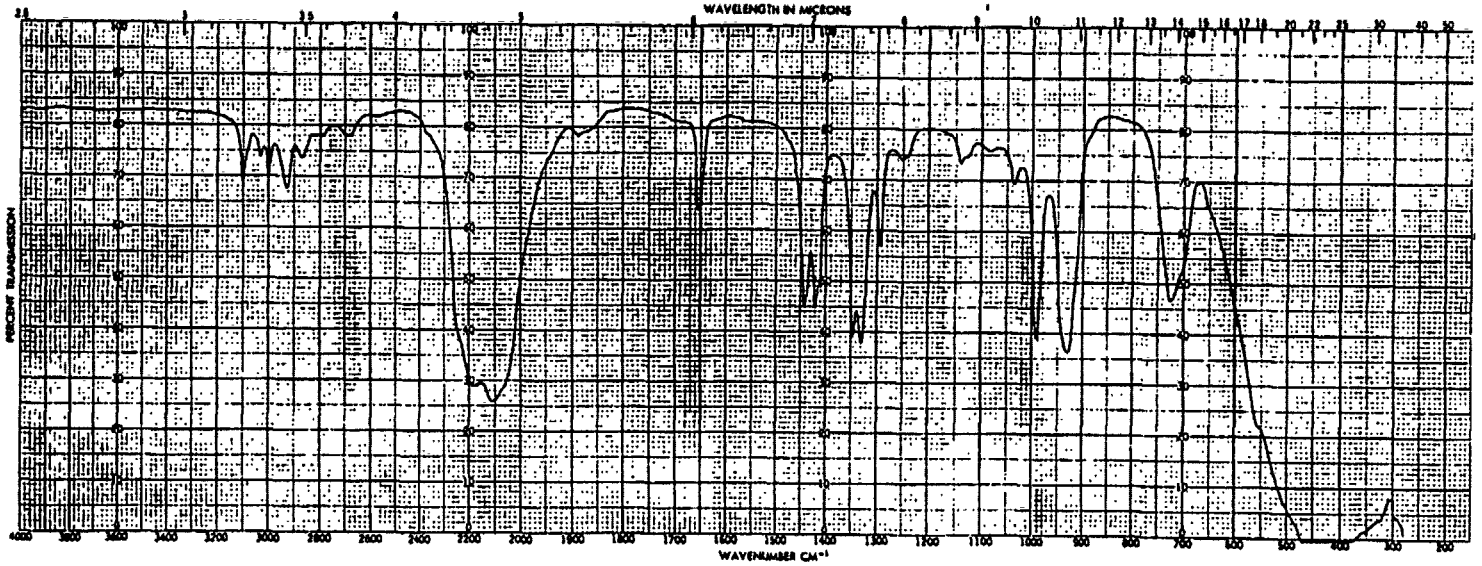

Figure 5. Infrared Absorption Spectrum of Allyl Isothiocyanate (Lot No. 532251)

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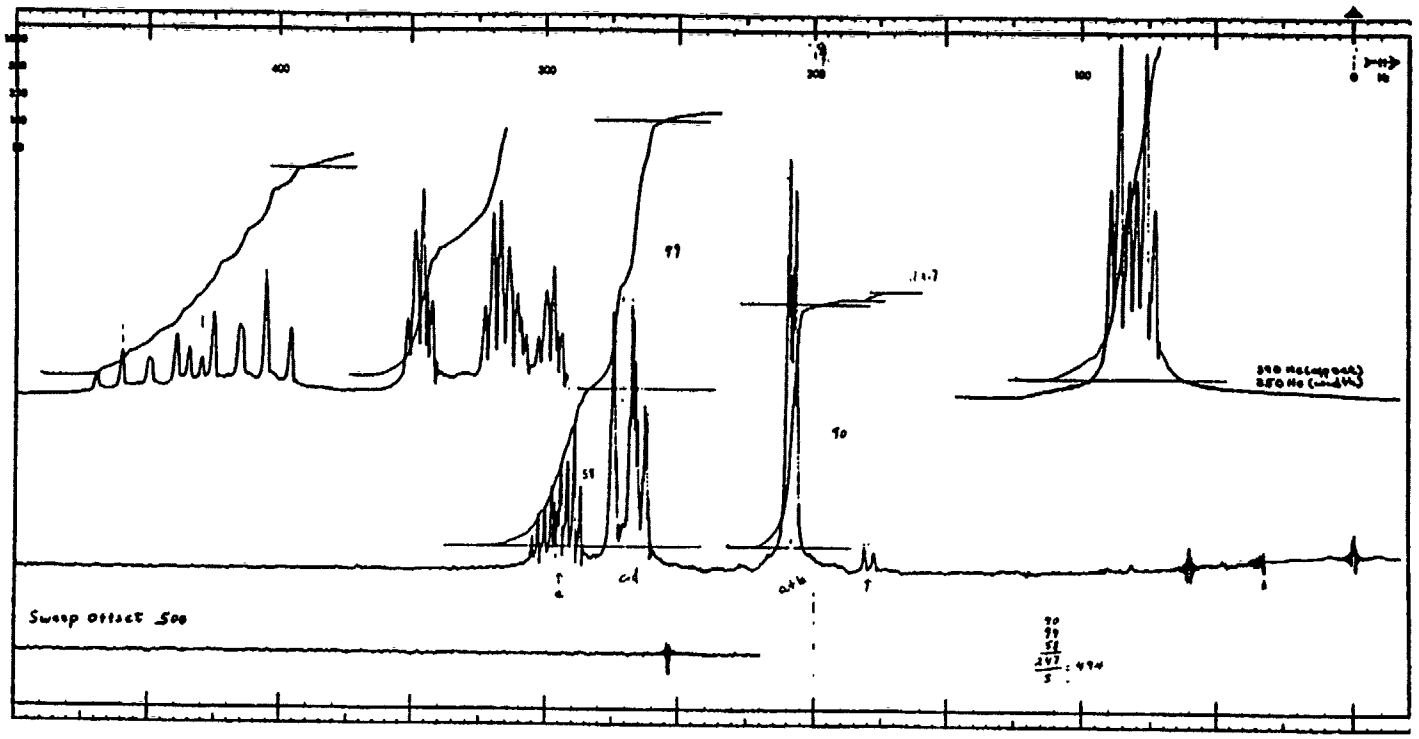

Figure 6. Nuclear Magnetic Resonance Spectrum of Allyl Isothiocyanate (Lot No. 532251)

APPENDIX F

ANALYSIS OF ALLYL ISOTHIOCYANATE IN CORN OIL FOR STABILITY OF ALLYL ISOTHIOCYANATE

A. PREPARATION OF SAMPLE AND STORAGE

A 26-μl aliquot of allyl isothiocyanate (26.90 mg) was placed in a 50-ml volumetric flask containing 50 ml corn oil, shaken, and placed in an ultrasonic vibrator bath for 30 seconds. The flask was stored at room temperature for 7 days with no effort made to protect the solution from light.

B. DILUTION AND ANALYSIS

1. Procedure

A 1.84-ml aliquot of the above stock solution (allyl isothiocyanate in corn oil) was pipetted into a small septum vial and 2 ml of anhydrous ethyl ether containing decane (15.63 mg decane in 50 ml ether) was added. The septum vial was sealed and mixed on a vortex mixer for 1 minute and placed in an ultrasonic vibrator bath for 2 minutes. The ether-corn oil mixture was analyzed by vapor-phase chromatography.

Note: Solvents which were immiscible with corn oil, such as alcohols, were not used due to their reactivity with allyl isothiocyanate. Therefore, dilution rather than extraction was used.

2. Instrumental Parameters

Instrument: Bendix 2500 with Hewlett-Packard 3380A automatic recorder/integrator
Detector: Flame ionization
Column: 20% SP2100/0.1% Carbowax 1500 on 100/120 Supelcoport,
1.8 m × 4 mm I.D., glass
Oven temperature: 90°C, isothermal
Inlet temperature: 130°C
Detector temperature: 285°C
Carrier gas: Nitrogen
Carrier flow rate: 50 cc/min
Sample injected: 5 μl

C. QUALITY ASSURANCE PROCEDURES

Analysis was performed in duplicate using decane as an internal standard. Linearity studies were done at two concentration levels (0.26 mg/ml and 0.13 mg/ml or 0.026% and 0.013%) to determine the relative weight response of compound versus internal standard (decane).

D. RESULTS

Day	Theoretical Percent (Chemical/Vehicle)	Determined Percent (Chemical/Vehicle)	Percent D/T × 100
0	0.02578	0.02578 ± 0.00081	100 ± 3
1	0.02578	0.02656 ± 0.00039	103 ± 2
2	0.02578	0.02480 ± 0.00031	96 ± 1
3	0.02578	0.02533 ± 0.00025	98 ± 1
4	0.02578	0.02455 ± 0.00084	95 ± 3
7	0.02578	0.02566	99.54

Retention time: Compound (4.7 min.), internal standard (11.7 min.)
Response of allyl isothiocyanate in corn oil versus that of allyl isothiocyanate in ether: 93.1 ± 0.3%

Linearity: $\text{RWR} \frac{\text{compound}}{\text{internal standard}} = 0.70 \pm 0.03$ at two concentration levels (0.026% and 0.013%).

E. CONCLUSION

The variation in the analysis is within the error of the method. Therefore, allyl isothiocynate is stable in corn oil at 0.05% concentration when stored at room temperature for 7 days without protection from light.

APPENDIX G

ANALYSIS OF ALLYL ISOTHIOCYANATE IN CORN OIL FOR CONCENTRATIONS OF ALLYL ISOTHIOCYANATE

Allyl isothiocyanate in corn oil mixtures was analyzed directly by vapor-phase chromatography. Extractions were not performed on the samples since corn oil does not interfere with the analysis. Gas chromatography conditions were as follows:

Column:	3% OV-17 on 80/100 Supelcoport, *1.8 m x 2 mm I.D., glass*
Detection:	Flame Ionization
Temperatures:	Inlet, 250°C Oven, 75°C, isothermal Detector, 275°C
Retention Time:	1.1 min.
Injection Size:	1 μl

There was no correction for work-up loss since samples were injected without any work-up. Reference samples of allyl isothiocyanate were prepared in corn oil and analyzed under the same conditions.

Results: See Table G1.

TABLE G1. ANALYSIS OF ALLYL ISOTHIOCYANATE IN CORN OIL FOR CONCENTRATIONS OF ALLYL ISOTHIOCYANATE

Date Mixed *(a)*	Used During Week of:	Concentration *(b)* of Allyl Isothiocyanate for Target Concentration of			
		0.12% (v/v)	0.24% (v/v)	0.25% (v/v)	0.50% (v/v)
04/10/78	04/11/78	0.10	0.23	0.25	0.48
05/05/78	05/06/78			0.25	0.48
06/07/78	06/08/78			0.25	0.48
07/05/78	07/06/78	0.12	0.24		
08/16/78	08/17/78			0.25	0.50
09/13/78	09/14/78	0.11	0.25		
10/11/78	10/12/78				0.50
11/09/78	11/10/78			0.24	
12/06/78	12/08/78				0.48
01/04/79	01/05/79			0.25	
02/01/79	02/02/79				0.47
03/01/79	03/02/79			0.25	
03/29/79	03/30/79				0.51
04/26/79	04/27/79			0.24	
05/24/79	05/25/79				0.53
06/21/79	06/22/79			0.24	
07/19/79	07/20/79				0.49
08/16/79	08/17/79			0.24	
09/13/79	09/14/79				0.46
10/11/79	10/12/79			0.24	
11/08/79	11/09/79				0.51
12/06/79	12/08/79			0.23	
01/03/80	01/04/80				0.51
02/01/80	02/02/80	0.11	0.26		
02/28/80	02/29/80			0.27	0.52
Mean (%)		0.11	0.25	0.25	0.49
Standard Deviation		0.01	0.01	0.01	0.02
Coefficient of variation (%)		9.1	4.0	4.0	2.0
Range (%)		0.10-0.12	0.23-0.26	0.23-0.27	0.46-0.53
Number of samples		4	4	13	14

(a) Start dates were March 1978 for rats and mice.
(b) The data presented are the average of duplicate analyses.

APPENDIX H

CUMULATIVE MEAN BODY WEIGHT CHANGE OF RATS AND MICE ADMINISTERED ALLYL ISOTHIOCYANATE BY GAVAGE IN THE CHRONIC STUDY

TABLE H1. CUMULATIVE MEAN BODY WEIGHT CHANGE (RELATIVE TO CONTROLS) OF RATS ADMINISTERED ALLYL ISOTHIOCYANATE BY GAVAGE

		Cumulative Mean Body Weight Change (grams)			Weight Change Relative to Controls *(a)* (Percent)	
	Week No.	Control	Low Dose	High Dose	Low Dose	High Dose
Males	0	133 *(b)*	134 *(b)*	133 *(b)*		
	5	115	115	108	0	- 6
	26	272	273	237	0	-13
	47	332	336	296	+ 1	-11
	79	337	345	324	+ 2	- 4
	104	317	326	298	+ 3	- 6
		450 *(c)*	460 *(c)*	431 *(c)*	+ 2 *(d)*	- 4 *(d)*
Females	0	99 *(b)*	102 *(b)*	100 *(b)*		
	5	48	51	50	+ 6	+ 4
	26	107	109	107	+ 2	0
	47	125	134	132	+ 7	+ 6
	79	166	184	180	+11	+ 8
	104	180	191	195	+ 6	+ 8
		279 *(c)*	293 *(c)*	295 *(c)*	+ 5 *(d)*	+ 6 *(d)*

(a) Weight change of the dosed group relative to that of the controls =

$$\frac{\text{Weight Change (Dosed Group)} - \text{Weight Change (Control Group)}}{\text{Weight Change (Control Group)}} \times 100$$

(b) Initial weight.

(c) Mean body weight at week 104.

(d) Mean body weight at week 104 relative to controls.

TABLE H2. CUMULATIVE MEAN BODY WEIGHT CHANGE (RELATIVE TO CONTROLS) OF MICE ADMINISTERED ALLYL ISOTHIOCYANATE BY GAVAGE

		Cumulative Mean Body Weight Change (grams)			Weight Change Relative to Controls *(a)* (Percent)	
	Week No.	Control	Low Dose	High Dose	Low Dose	High Dose
Males	0	22 *(b)*	23 *(b)*	22 *(b)*		
	5	7	6	6	-14	-14
	26	20	19	21	- 5	+ 5
	47	26	23	28	-12	+ 8
	79	28	27	32	- 4	+14
	104	26	23	27	-12	+ 4
		48 *(c)*	46 *(c)*	49 *(c)*	- 4 *(d)*	+ 2 *(d)*
Females	0	17 *(b)*	18 *(b)*	18 *(b)*		
	5	7	5	5	-29	-29
	26	11	10	11	- 9	0
	47	14	13	16	- 7	+14
	79	18	19	19	+ 6	+ 6
	104	20	18	18	-10	-10
		37 *(c)*	36 *(c)*	36 *(c)*	- 3 *(d)*	- 3 *(d)*

(a) Weight change of the dosed group relative to that of the controls =

$$\frac{\text{Weight Change (Dosed Group)} - \text{Weight Change (Control Group)}}{\text{Weight Change (Control Group)}} \times 100$$

(b) Initial weight.

(c) Mean body weight at week 104.

(d) Mean body weight at week 104 relative to controls.

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October 1982

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Consulting Toxicologist

Dr. Hobson has over 20 years of experience as a Toxicologist evaluating the exposure, effects and risks posed by pesticides and industrial chemicals to human health and the environment. He has a B.Sc. in Biology, a M.Sc. in Toxicology, and a Ph.D. in Biology. He is Board Certified in General Toxicology. Dr. Hobson has extensive experience managing professional staff with responsibilities for testing programs in support of existing and new chemical product registrations under FIFRA, and regulation under TSCA, FDA, and State, EU, and Canadian laws. He has held senior-level positions with two major chemical Companies, FMC Corporation and Rhône-Poulenc, and two industry-focused consulting firms, Technology Sciences Group Inc. and ARCADIS Geraghty & Miller, Inc. Dr. Hobson is currently President of MorningStar Consulting, Inc. an independent consulting firm providing Scientific and Regulatory Support Services to industrial clients.

Dr. Hobson has teamed with corporate, business and registration staff, and trade association task groups, in developing programs to address stewardship and regulatory concerns, defend current registrations, and obtain new product registrations. Dr. Hobson has designed, managed, and interpreted the results of toxicology and environmental toxicology programs summarizing results, conducting risk assessments, contributing to development of regulatory strategies, and interpreting and presenting program results to business teams, task groups, management, and regulatory agencies. He has extensive experience as liaison with chemical companies, industry trade associations, contract laboratories and regulatory agencies in the United States, Canada and Europe. Dr. Hobson has served as an expert witness and provided litigation support in cases relating to chemical contamination and impact on natural and commercial resources.

Areas of Expertise:

- Ecological and Human Health Risk Assessment
- Toxicology and Environmental Toxicology
- Board Certified in General Toxicology
- Pesticide registrations and product defense
- Chemical regulations (FIFRA, , TSCA, FDA, EU, and Canada) for pesticides, new and existing (high production volume, HPV) chemicals, veterinary drugs and food additives.
- Chemical-specific environmental assessments and risk assessments
- Liaison with regulators including those representing state, Federal, and foreign authorities.
- Extensive experience working with trade associations and task groups
- Study design, oversight, and interpretation.
- Laboratory Programs:
  - Toxicology: Acute, repeated dose, developmental toxicity, and *in vivo* and *in vitro* genotoxicity.
  - Ecotoxicology: Acute and chronic studies with aquatic and terrestrial species, and plants
  - Physicochemical properties and environmental fate chemistry
- Field and simulated-field studies:
  - Aquatic and terrestrial ecosystems

Education

Ph.D., University of Kentucky, Biology, 1986

M.Sc., University of Arizona Toxicology, 1981

B.Sc., Roanoke College, Biology, 1977

Professional Certifications

Diplomate of the American Board of Toxicology (1989,1994,1999)

Professional Associations

Society of Environmental Toxicology and Chemistry

Society of Toxicology

Technology Sciences Group Inc.

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Dr. Gary Burin is an internationally recognized expert in toxicology with twenty-five years of experience in the design, interpretation and use of toxicity studies. He consults on matters concerning pharmaceuticals, pesticides, food additives, veterinary drugs, industrial chemicals and consumer products and has extensive experience in working with the U.S.EPA and FDA, and with similar agencies in the European Union, Canada and the State of California. Dr. Burin has served on numerous agency, inter-Agency and international workgroups and expert panels and as an expert witness in public hearings and litigation.

During his 13 years at the EPA, Dr. Burin served as Section Head in the Health Effects Division, Manager of the Developmental and Reproductive Toxicity Peer Review Committee, Executive Secretary of the FIFRA Scientific Advisory Panel, and as a member of the Carcinogenicity Peer Review Committee and the Reference Dose Committee of the Office of Pesticide Programs. He received the Silver Medal from the U.S. EPA Administrator for his work in coordinating the risk assessment of ethylene dibromide and three Bronze medals for other significant contributions to the work of EPA.

While on the staff of the International Programme on Chemical Safety at the World Health Organization in Geneva, Switzerland, Dr. Burin managed and participated in expert working groups on the evaluation of potential health effects of pesticides, food additives, veterinary drugs, consumer products and drinking water contaminants, including the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) and the Joint FAO/WHO Expert Committee on Food Additives and Contaminants (JECFA). He managed the revision of the WHO Guidelines for Drinking Water Quality, an effort that spanned several years and included the evaluation of over 300 chemicals. Dr. Burin has authored numerous publications in the areas of toxicology and risk assessment. His publications include the World Health Organization (WHO) document "Environmental Health Criteria Document 104 - Principles for the Toxicological Assessment of Pesticide Residues in Food" and the EPA Standard Evaluation Procedures document that provides guidance for the evaluation of chronic, reproductive and developmental toxicity studies.

Dr. Burin has taught at the Universities of Sienna, Italy, and Surrey, England and is an Adjunct Associate Professor in the Division of Occupational Medicine at the George Washington University Medical School where he lectures in toxicology, risk assessment and issues in environmental health.

Dr. Burin earned his B.S. in Biology and M.P.H. (concentration in toxicology) from the University of Michigan and his Ph.D. in Biology, with a Pharmacology minor, from the George Washington University. He has been a Diplomate of the American Board of Toxicology since 1985 and is a member of the Society of Toxicology, the International Society for Regulatory Toxicology and Pharmacology and the Teratology Society.

Submission End