

## 2,4-Hexadienal

### CHEMICAL IDENTIFICATION:

Structure:	CH <sub>3</sub> -CH=CH-CH=CH-CHO
Molecular Formula:	C <sub>6</sub> H <sub>8</sub> O
Molecular Weight:	96.13
CAS Registry Number:	142-83-6
Other CAS Nos.:	53398-76-8 ( <i>trans, cis</i> -2,4-Hx) 80466-34-8 (mixed isomers)
Chemical Abstr. Name:	2,4-Hexadienal,(E,E)-(9CI)
Synonyms/Trade Names:	Sorbic aldehyde Sorbaldehyde 2-propyleneacrolein 1,3-pentadiene-1-carboxaldehyde <i>trans,trans</i> -2,4-hexadienal all- <i>trans</i> -2,4-hexadienal hexa-2,4-dienal 2,4-hexadien-1-al 2,4-Hx

### CHEMICAL AND PHYSICAL PROPERTIES

2,4-Hexadienal (2,4-Hx) is a colorless (yellowish) liquid with pungent sweet-green citrusy odor (Ford *et al.*, 1988). It is insoluble in water, soluble in alcohol (Bedoukian, 1993), and reacts with strong oxidizing and reducing agents. It has a boiling point of 64°C (147°F, Lancaster Synthesis, Inc., 1991), a specific gravity of 0.871, a vapor density of >1, and a vapor

pressure of 1.6 mm Hg at 20° C. Its refractive index is 1.535 and flash point is 130°F (67°C, Aldrich, 1991; 1992). 2,4-Hx is available from Aldrich Chemical Co. in 95% purity. No information on impurities is available.

## NOMINATION RATIONALE

2,4-Hx is a natural constituent of meat, vegetable, and fish oils. It is one of the lipid peroxidation products of polyunsaturated oils which undergoes auto-oxidation especially during storage (Snyder *et al.*, 1985) and have been implicated in the development of off or tainted flavor. 2,4-Hx is also used as a food additive or flavoring agent.

Lipid hydroperoxides have been shown to give rise to low intracellular levels of  $\alpha,\beta$ -unsaturated aldehydes, including 2,4-Hx and 2,4-decadienal (2,4-De). Some of the  $\alpha,\beta$ -unsaturated aldehydes have been shown to be reactive with DNA (Frankel *et al.*, 1987). Ingested lipid oxidation products and oxidized fats have been reported to cause increased excretion of mutagens, cellular injury to liver and kidneys, increased cell proliferation in the gastro-intestinal tract, and other non-specific tissue injury and irritation effects resulting from induced oxidative stress.

There is an overall lack of data generated from testing of dienals for either carcinogenicity or mutagenicity and a lack of evidence specifically relating exposures to cancer in humans. The role of consumed oxidized oils in gastro-intestinal carcinogenesis including the effects of oral intake of different doses of various biologically active compounds present in heated oils, effects of oxidative stress induced by chronic consumption of repeatedly heated oils, as well as interactions with other modulating dietary factors, including both macro- and micro-nutrients have not been investigated (Hageman *et al.*, 1991). Testing of 2,4-Hx and 2,4-De for toxicity and carcinogenicity would help mitigate the data gap.

The CSWG on 9/18/92 recommended with high priority evaluation of the

metabolism, distribution, mutagenesis, and carcinogenesis of 2,4-Hx with 2,4-De as a matched pair. These commonly found auto-oxidation products of unsaturated fats appear to be inactive in most standard mutagenicity prescreens, both show signs of mutagenic activity in tests using TA104, or by increasing the bacterial density, or by adding glutathione to the mix (Eder et al., 1992).

## PRODUCTION AND PRODUCERS

2,4-Hx is prepared by condensation of acetaldehyde (Keller *et al.*, 1983). 2,4-Hx is available for sale from Aldrich Chemical Co., American Tokyo Kasei, Chem Service, Inc., Crescent Chemical Co., Pfaltz & Bauer, Inc., and Lancaster Synthesis, Inc. Current production levels are not available but the FDA's Priority-based Assessment of Food Additives (PAFA) database contains the following data (FDA, 1992):

<u>Year</u>	<u># of Reporting Companies</u>	<u>Market Disappearance Rate,lb/yr</u>
1970	5	43
1975	-	6
1976	5	7
1982	7	26
1987	6	3.3

## USE PATTERN

2,4-Hx is used as a flavoring agent in the manufacture of aroma chemical, 3,5,7-nonatrien-2-one. It is a chemical intermediate used in various organic synthetic reactions. 2,4-Hx has been used as the starting material for the manufacture of sorbic acid (Keller *et al.*, 1983) widely used as a food preservative.

2,4-Hx is also used as chemical intermediate in the manufacture of polymethine dyes (Sturmer and Diehl, 1982), pharmaceutical intermediate

for manufacture of mitomycins and antihypercholesteremics, corrosion inhibitor for steel in oil field operations (Growcock *et al.*, 1989), monomer reacted with a silane comonomer in polyalkenyloxysilane polymer manufacture, fumigant against larvae of the Caribbean fruit fly, reducing agent in agricultural research studies for photoreduction of nitrophenyl ether herbicides to nitro radical anions

### **Environmental Occurrence**

2,4-Hx occurs naturally as an autoxidation product of unsaturated fatty acids, *e.g.* linoleic acid. It has been identified in numerous oxidized glyceridic oils, including canola (low erucic acid rapeseed) oil, soybean oil, cottonseed oil, sunflower oil, sesame oil and palm oil. In addition it has been detected in the essential oils of lovage, thyme leaf and dill and in solid alfalfa extract. It has been associated with rancidity and off-flavor in fish, including farm raised catfish, Gulf of Mexico menhaden and Upper Wisconsin River walleye and northern pike (Heil and Lindsay, 1988). It occurs naturally in a variety of plant products, including cotton, tomatoes with poor flavor, mango, kiwi and Chinese quince, and is a component of tobacco leaf and tobacco smoke volatiles (Takeoka *et al.*, 1986; Weeks *et al.*, 1989; Wright and Harris, 1985; Zeringue and McCormick, 1989). It has been detected as a volatile of piled (rather than picked) Toyama Kurocha tea processed in Japan. 2,4-Hx was not found in fresh steamed or fermented tea leaves, but was reported at a concentration of 0.4 mg/100 mg in the solar dried product and 0.2 mg/100 mg in the product stored for 1 year (Kawakami and Shibamoto, 1991).

2,4-Hx was identified as one of several ring fragmentation products present in polluted urban air (Dumdei *et al.*, 1988). 2,4-Hx was also identified as a low-level carbonyl impurity in commercial ethanol, even in distilled premium grades (Sherman and Kavasmaneck, 1980).

The presence of 2,4-Hx in mussels, oysters at 35 mg/gm (35 ppb) and clams at 7.5 mg/gm (7.5 ppb) from Lake Pontchartrain has been attributed

to water pollution by VOCs (Ferrario *et al.*, 1985). 2,4-Hx has also been cited in a recent Russian review of aldehydic environmental pollutants.

### **Human Exposure**

2,4-Hx has been detected in tobacco and tobacco smoke (Florin *et al.*, 1980; Pettersson *et al.*, 1980) and is present in seafood (Ferrario *et al.*, 1985), oxidized edible fats and oils, heated oils for food frying, and rancid or off-flavored fish oils (Przybylski and Hougen, 1989; Selke and Rohwedder, 1983; Suzuki and Bailey, 1985; White and Hammond, 1983). Other consumed products in which it has been detected include meat fat, cow's milk fat, potato chips, bread crust, dried and stored piled tea, herbs and spices, and tropical fruits.

According to the Flavor & Extract Manufacturers Association total yearly consumption of 2,4-Hx is 2.48 kg of which 0.9 kg (36%) is used as a flavor ingredient and 1.58 kg is consumed as a component of food (FEMA No. 3429). According to Ford, *et al.* (1988) the reported maximum concentration of this chemical as an ingredient in consumer products is 0.1%.

### **Regulatory Status**

2,4-Hx is listed in EPA's TSCA inventory. No standards or guidelines have been set for occupational exposures to or environmental levels of 2,4-Hx. The American Conference of Governmental Industrial Hygienists (ACGIH) has not adopted a TLV/TWA for this compound.

2,4-Hx was given GRAS (generally recognized as safe) status after a review of flavoring ingredients and food additives by the Flavoring Extract Manufacturers' Association (FEMA) and was listed in 1981 by the Council of Europe as a flavoring substance that may be added to food (Ford *et al.*, 1988).

## Toxicity

$\alpha,\beta$ -unsaturated aldehydes are direct-acting alkylating agents capable of covalent binding without prior metabolism to cellular nucleophilic groups (Eder et al., 1993). The potential for being toxic and/or capable of modifying cellular processes is inherently present. Very little is known about the in vivo effects of 2,4-Hx, their target tissues, and mechanisms of toxicity.

Human Data: No information was found in the literature associating 2,4-Hx with a cancer risk in humans. Health hazard advisory information in the Aldrich MSDS includes severe irritant and toxic effects following inhalation or dermal absorption, with tissue destruction of mucous membranes of the upper respiratory tract, eyes and skin. Ford *et al.* (1988) cited the results of dermal 48-hr closed patch tests carried out at a concentration of 1% 2,4-Hx in petroleum on the backs of 59 volunteers in which no irritation but 1 case of sensitization was reported.

High rates of colon cancer in the northeastern U.S. have been associated with lifestyle, especially diet. Furthermore, it has been postulated that the high colon cancer incidence may be linked to a high fat diet or one low in fruits and vegetables and low in vitamin A (Urbany, 1992). Free radicals, singlet oxygen and other reactive species formed in the peroxidation of lipids are considered biologically harmful and are implicated in cellular damage and cancer (Frankel *et al.*, 1987). The difficulties of assessing the cancer risk from multiple low level exposures to a wide variety of food toxicants is daunting. Marnett *et al.* (1985) have postulated that, "since carbonyl compounds are widely distributed in foods, are generated during cellular metabolism, and are present in body fluids, they make a significant contribution to the risk of human cancer."

Animal Data: The feeding of lipid oxidation products and oxidized fats has been reported to cause adverse biological effects in laboratory animals, including growth retardation, teratogenicity, tissue damage and increased

weights of the liver and kidneys (Alexander et al., 1987; Izaki et al., 1984; Kanazawa et al., 1985; 1986), cellular damage to the testes and epididymides, increased peroxidation of membrane and tissue lipids and induction of cytochrome P450 activities in the liver and colon (Crawford and Wheeler, 1983). No information was found in the literature associating 2,4-Hx with a cancer risk in animals.

The following information is recorded in RTECS database (NLM, 1992):

Oral rat LD50:	300 mg/kg
Dermal rabbit LD50:	270 mg/kg
Dermal guinea pig LD10:	2,500 mg/kg
Dermal guinea pig LD50:	5,000 mg/kg
Inhalation rat LC10:	2,000 ppm/4H
Dermal rabbit:	Severe irritant
Ocular rabbit:	Severe irritant
Dermal guinea pig:	Severe irritant and sensitizer

The irritating effect of 2,4-Hx may cause cellular injury and cell proliferation in the esophageal tissues and other parts of the alimentary tract following oral administration.

### Mutagenicity

Florin *et al.* (1980) reported that 2,4-Hx was non-mutagenic when tested at a concentration of 3  $\mu$ M/plate in Ames/*Salmonella* tester strains TA98, TA100, TA1535, and TA1537. In the NCI/DCE Short-Term Test Program, 2,4-Hx was negative in the Ames/*Salmonella* mutagenicity assay using tester strains TA98, TA100, TA1535, TA1537, AND TA1538 when tested at doses up to 10,000  $\mu$ g/plate with and without metabolic activation. In the mouse lymphoma L5178Y TK+/- assay, 2,4-Hx was positive when tested at doses ranging from 3.1 to 0.6  $\mu$ g/ml without metabolic activation but was negative when tested at doses ranging from 74 to 23  $\mu$ g/ml with activation (NCI, 1992a).

Marnett *et al.* (1985) reported that 2,4-Hx tested positive for mutagenicity when measured in a liquid pre-incubation procedure in an Ames/*Salmonella* assay using strain TA104, with a result of 960 revertants/ $\mu$ M. No mutagenicity was observed with strain TA102. These authors found that 2,4-Hx was cytotoxic but the toxicity could be reduced by addition of glutathione. Both TA102 and TA104 are base substitution strains developed to detect peroxides and other oxidants. TA104 carries a nonsense mutation (-TAA-) at the site of reversion that is present in single copy on the chromosome and TA102 contains the same histidine mutation on a multicopy plasmid. 2,4-Hx was also tested positive in TA100 without S9 in a modified preincubation Ames test (Eder *et al.*, 1992; 1993). Positive results were obtained by increasing the bacterial number (3-fold) and by using glutathione as a chase. (Note: the molecular mechanisms leading to backmutation in strain TA104, which carries the his G428 mutation cannot be directly compared with those of the his G46 strains).

Eder *et al.* (1993) demonstrated that toxicity of 2,4-Hx interferes with mutagenicity testing in *Salmonella* strain TA100. But with higher cell numbers toxicity can be partly compensated and mutagenicity of 2,4-Hx without S9 was demonstrated in preincubation assays. In SOS chromotest using *E. coli* strain PQ37, 2,4-De was positive when ethanol was used as solvent, but was negative when dimethylsulfoxide was used (Eder *et al.*, 1993). 2,4-Hx induced DNA strand breaks at high dose (500  $\mu$ mole) as measured in the alkaline elution technique when cytotoxicity was also observed. 2,4-Hx also interacted with guanine and formed DNA adducts (1,2-cyclic deoxyguanosine adduct and 7,8-cyclic guanosine adduct) (Eder *et al.*, 1993)

Strand breaks can be due to direct DNA interaction but also to cell death-associated release of endonucleolytic enzymes. Brambila *et al.* (1986) found that lipid peroxidation products can remain associated with the lipid core of the endoplasmic reticulum and that they can diffuse into the cell nucleus without encountering cytosolic detoxifying enzymes. Thus, relatively high concentrations can interact with DNA *in vivo*.



$\alpha,\beta$ -unsaturated aldehydes have been shown to cause unscheduled DNA synthesis in primary cultures of rat hepatocytes (Griffin et al., 1986), DNA fragmentation and sister chromatid exchange in Chinese hamster ovary cells (Brambila et al., 1986), and inhibition of the DNA repair enzyme O<sup>6</sup>-methylguanine-DNA methyltransferase (Krokan et al., 1985).

## **DISPOSITION**

Hydroperoxides and low-molecular weight secondary oxidation products of linoleic acid have been reported to be readily absorbed. After administration of a single dose of <sup>14</sup>C-labelled methyl linoleate hydroperoxides by gastric intubation to rats, up to 80% of the dose were recovered mainly in tissues of the stomach and liver (Bergan and Draper, 1971, Kanazawa et al., 1985; Oarada et al., 1986). Studies of dienals have not been conducted.

Aldehydes are principally metabolized in the liver. Alcohol dehydrogenase is capable of catalyzing the metabolic reduction of aldehydes to primary alcohols in the reversal of the metabolic reaction involving the oxidation of primary alcohols to aldehydes. In mammals, however, this is not necessarily found to occur, since aldehydes are preferentially oxidized to the corresponding acids. The acids formed as major metabolites of aldehydes may then be excreted or form conjugates which are excreted. Aldehyde dehydrogenase, with NADH as cofactor, has been shown to dehydrogenate short chain aliphatic aldehydes as well as aromatic aldehydes (McMahon, 1982; Esterbauer et al., 1985).

## **Biological Effects**

While free radical and lipoperoxides produce direct damage at the cell structures where they are produced, aldehydes are more diffusible and long-lived and may induce damage at distant sites.

### **1. Interact with DNA**

$\alpha,\beta$ -Unsaturated aldehydes ingested or produced endogenously as a result of lipid peroxidation during normal metabolic process or induced by exogenous chemicals (CCl<sub>4</sub>, CHCl<sub>3</sub>, DDT, PCB, etc) are strong electrophilic reagents and react readily with nucleophilic groups. Thus, 2,4-Hx is expected to interact with DNA.

Eder et al. (1993) demonstrated that members of the  $\beta$ -alkyl-substituted acrolein congeners (pentenal, hexenal, 3,3-dimethylacrolein) form 1,N<sup>2</sup>-cyclic adducts and 7,8-cyclic adducts with deoxyguanosine in vitro similar to those observed with crotoaldehyde. Their data showed that 2,4-Hx would formed similar adducts with deoxyguanosine although they could not isolate the adducts in sufficient quantity. They postulated that since these adducts are premutagenic DNA lesions and that crotonaldehyde is carcinogenic, the  $\beta$ -alkyl-substituted acrolein congeners are considered carcinogenic.

A fluorescence is generated when DNA interacts with a lipid degradation product. Using this technique Frankel et al. (1987) demonstrated that 2,4-De is readily interact with calf thymus DNA in vitro in the presence of ferric chloride and ascorbic acid.

## 2. Interact with glutathione

Administration of acrolein via the oral route cause a dose-dependent depletion of glutathione in the liver. GSH conjugation of acrolein mediated by glutathione transferases is considered a detoxification mechanism. But evidence has been shown that conjugates may transport the chemical to be activated at a new site (Witz, 1989). Thus, the biological fate of thiol conjugates of  $\alpha,\beta$ -unsaturated aldehydes remains to be explored.

## 3. Inhibition of cell proliferation (Carcinostatic/therapeutic /Inhibition of tumor cell growth)

Aldehydes have been shown to inhibit cell proliferation. (tumor cells are

more sensitive to aldehydes than normal cells, due to aldehyde dehydrogenase activity). The mechanism may involve interaction with tubulin, inhibition of polyamine metabolism, adenylate cyclase, lysosomes (Dianzani, 1982).

The sulfhydryl reactivity of  $\alpha,\beta$ -unsaturated aldehydes may play a role in carcinostatic action, e.g. 1:2crotonaldehyde-cysteine adduct and the 1:1 trans-4-hydroxypentenal-cysteine adduct. The action is due to the reactivity of the double bond in interaction with sulfhydryls. The aldehydes may react with enzymes containing the essential sulfhydryl groups.

2,4-Hx was investigated for use as a food preservative but was found inactive in retarding the growth of food molds (fungi) (Troller and Olsen, 1967). On the other hand, Gueldner *et al.* (1985) reported that 2,4-Hx, occurring naturally in corn ears, appeared to act as a natural mycostatic insecticide, inhibiting growth of *Aspergillus flavus*.

#### 4. Inhibition of enzymes (membrane bound enzymes?)

DNA repair enzyme O<sup>6</sup>-methylguanine-DNA methyl transferase by forming cysteine-trans-4-hydroxynonenal adduct.

The effect of  $\alpha,\beta$ -unsaturated aldehydes on rat liver microsomal glucose 6-phosphatase has been studied. Depending on the chain-length, the Michaelis constant,  $K_m$  and the maximal rate of reaction,  $V$ , were affected. However, both 2,4-Hx and 2, 4-De did not alter the kinetic constants and  $K_m$  of the enzyme. The lack of effects may be attributed to the rather rigid planar structure around the 3 conjugated double bonds giving rise to a severe sterical hindrance at the  $\beta$ - and  $\delta$ -carbon atoms (Jorgensen *et al.*, 1992).

Other enzymes known to be inhibited: cytochrome P-450  
aminopyrine demethylase  
adenylate cyclase

## 5. Cytotoxicity

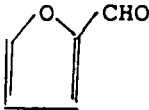
Cytotoxic effect of  $\alpha,\beta$ -unsaturated aldehydes may be due to inhibition of superoxide anion radical production in macrophage and neutrophils or decrease in membrane lipid fluidity (see Witz, 1989). For examples, in a study of the effects of tobacco smoke components, Thelestam *et al.* (1980) found that 2,4-Hx at a concentration of 25 mM caused an increase of 20% in the membrane permeability of human lung fibroblasts incubated for 30 minutes. 2,4-Hx at a concentration of 0.01 mM in ethanol inhibited murine Ascites sarcoma BP8 cells 44% while 0.1 and 1.0 mM concentrations were 100% cytotoxic (Pilloti *et al.*, 1975). 2,4-Hx at a concentration of 0.1 mM inhibited noradrenaline induced oxidative metabolism in isolated hamster brown-fat cells and at 1 mM caused 100% inhibition (Pettersson *et al.*, 1980). A concentration of 5 mM 2,4-Hx applied to chicken embryo tracheal organ cultures for 6 minutes caused complete cessation of ciliary activity (Pettersson, *et al.*, 1980).

6.  $\alpha,\beta$ -Unsaturated aldehydes, including 2,4-Hx, react with thiobarbituric acid (TBA) forming a reddish pigment which is the basis of human clinical lipid peroxidation analysis. Kosugi *et al.* (1988) observed synergism between 2,4-alkadienals and other aldehydes and hydroperoxides as evidenced by the intensity of the red pigment formation.

## Structure/Activity Relationships

The Interagency Testing Committee (ITC) has classified chemicals containing the closely related substructure,  $\alpha,\beta$ -unsaturated aldehyde, as a group of chemicals likely to be associated with adverse health and ecological effects. Their concern for potential health effects resulting from exposures to this class of chemicals includes oncogenicity, mutagenicity and membrane irritation.

The known cancer risk of  $\alpha,\beta$ -unsaturated aldehydes in the diet is summarized below:

Chemicals	Mutagenicity	Carcinogenicity	Ref.
Acrolein $\text{CH}_2=\text{CH}-\text{CHO}$	-, +w	Deferred	NTP
2-Chloroacrolein		+	Robinson et al.
2-Bromoacrolein		+	Robinson et al.
Malondialdehyde $\text{HOC}-\text{CH}=\text{CH}-\text{CHO}$	-	$\text{MR}^+\text{FR}^+\text{MM}^-\text{FM}^-$	NTP
Crotonaldehyde $\text{CH}_3-\text{CH}=\text{CH}-\text{CHO}$	+	+	Chung et al., 1986 (in drinking water)
Furfural 	?	$\text{MR}^+\text{FR}^-\text{MM}^+\text{FM}^+$	NTP
Citral $\text{H}_3\text{C}-\underset{\text{CH}_3}{\text{C}}=\text{CH}-(\text{CH}_2)_2-\underset{\text{CH}_3}{\text{C}}=\text{CH}-\text{CHO}$	-	on going	NTP
vanillin	-		NTP
glycidaldehyde	+		NTP
benzaldehyde	-		Animal carcinogen, IARC
2,4,5-Trimethoxybenzaldehyde	-	$\text{MR}^-\text{FR}^-\text{MM}^+\text{FM}^+$	NTP
cinnamaldehyde $\text{C}_6\text{H}_5-\text{CH}=\text{CH}-\text{CHO}$	?	on going	NTP
Trimethoxycinnamaldehyde	+		
anisaldehyde			
Fecapentaenes	+		Assoc. human colorectal CA Vertegaal et al., 1992
2,4-hexadienal $\text{CH}_3-\text{CH}=\text{CH}-\text{CH}=\text{CH}-\text{CHO}$	+		Eder et al., 1992
2,4-Decadienal $\text{C}_5\text{H}_{11}-\text{CH}=\text{CH}-\text{CH}=\text{CH}-\text{CHO}$	+		Marnett et al., 1985 Eder et al., 1993

MR: male rats; FR: female rats; MM: male mice, FM: female mice

The central structural feature of an  $\alpha,\beta$ -unsaturated aldehyde is its bifunctional group, the carbon-carbon double bond conjugated with the aldehydic carbonyl group.  $\alpha,\beta$ -unsaturated aldehydes are direct-acting alkylating agents capable of covalent binding without prior metabolism to cellular nucleophilic groups, the potential for being toxic and/or capable of modifying cellular processes is inherently present in every member of this class (Witz, 1989).

In studying mutagenesis of  $\beta$ -alkyl substituted acrolein congeners (crotonaldehyde, trans-2-pentenal, trans-2-hexenal, 2,4-hexadienal, and trans-2-heptenal) Eder et al. (1992) observed that toxicity increases as a function of both chain length and lipophilicity:

1. Increasing chain length increases toxicity and decreases mutagenicity  
This effect may be explained by increasing toxicity as mutagenic responses cannot be observed in some cases because of competing cytotoxicity (Eder et al., 1992).
2. Increasing  $\beta$ -chain length increases lipophilicity and toxicity.  
The dependence of toxicity on increasing  $\beta$ -chain length can be ascribed to increasing lipophilicity which allows a better penetration through the cell membrane.
3. The conjugated double bond in the  $\beta$ -alkyl chain (2,4-Hx) increases mutagenesis; this is also in accordance with the hypothesis that lipophilicity determines toxicity. (In in vitro studies only, what about in vivo?)
4. Decrease in mutagenicity with increasing molecular weight.  
This is due to decrease in reactivity of  $\alpha,\beta$ -unsaturated carbonyl compounds towards nucleosides and nucleotides with increase molecular weight (Eder et al., 1993).

## **RECOMMENDATIONS**

Since 2,4-Hx is mutagenic and cytotoxic and it has been shown that 2,4-Hx induced DNA strand breaks and forming adducts with guanine, its potential to induce cancer is high. However, it remains to be shown whether it can induce these events in vivo and ultimately cancer. The chemical is produced endogenously and humans are exposed to it mainly by mouth through food. The organism normally has a protective mechanism in detoxifying the chemical. In order to induce toxicity and carcinogenicity, the chemical has to be administered in large doses to overwhelm the natural detoxifying mechanism. The following studies are recommended:

1. Disposition studies be conducted. There is no information on absorption, toxicokinetics, metabolism, distribution, and excretion of 2,4-De in the literature.
2. Gavage studies in 0.5% methyl cellulose be conducted: 14-day and 90-day dose range finding studies and 2-year carcinogenesis studies.
3. Stability study be conducted: Stability of 2,4-De in 0.5% methyl cellulose.
4. The role of glutathione and thiols and vitamins C and E be investigated. It has been shown in vitro that the presence of glutathione reduces toxicity of a,b-unsaturated carbonyl compounds. Administration of 2,4-De may deplete liver glutathione and blood vitamins C and E.
5. Cell proliferation studies be conducted. Increased proliferative activity in the esophagus have been shown to occur after feeding with heated polyunsaturated oil (Hageman et al., 1991). Bull et al. (1984; 1988) and Craven et al. (1987) also showed that hydroperoxides of linoleic acids as well as other oxidative degradation products and metabolites of polyunsaturated fatty acids enhanced cell proliferation in the colonic tissue. Therefore, esophagus, stomach, colon, small and large intestine, mammary gland, kidney, and liver shall be processed for possible PCNA staining.

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