NATIONAL TOXICOLOGY PROGRAM

EXECUTIVE SUMMARY OF SAFETY AND TOXICITY INFORMATION

ETHOXYQUIN

CAS Number 91-53-2

August 21, 1990

Submitted to:

NATIONAL TOXICOLOGY PROGRAM

Submitted by:

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Chemical Evaluation Committee Draft Report

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OVERVIEW[1]

Nomination History: *Ethoxyquin was nominated for carcinogenicity testing by the Food and Drug Administration (FDA) in 1990. The nomination of ethoxyquin was based on the increased use and high levels of ethoxyquin (150 ppm) in animal feed. The FDA also noted general uncertainty concerning the potential toxicity of ethoxyquin which has been found to induce kidney damage in rats.*

Chemical and Physical Properties: *Ethoxyquin is a dark-colored liquid, varying in color from yellow to brown, with a mercaptan-like odor. The boiling point of ethoxyquin is 123°C (257°F). This compound is insoluble in water and soluble in animal and vegetable fats and oils.*

Production/Uses/Exposure: *The primary manufacturer of ethoxyquin in the United States is Monsanto Industrial Chemical Company, under the tradename Santoquin. Up to 1,000,000 pounds of ethoxyquin were reportedly manufactured in the U.S. in 1977 by three plants. No other data on production was available. Ethoxyquin is used primarily as an antioxidant preservative in animal feed and dehydrated forage crops, and as an anti-scald agent in pears and apples. It is also used as a color preservative in spices, and as an anti-degradation agent for rubber. Limited data was found on occupational exposure to ethoxyquin. However, data from the National Occupational Exposure Survey indicate that 6,913 male employees were potentially exposed to ethoxyquin between 1981 and 1983. The FDA has set a residue tolerance of 0.5 ppm ethoxyquin for meat and meat by-products, and a residue level of 3.0 ppm for uncooked fat and poultry, and for pears and apples. There is no OSHA permissible exposure limit for ethoxyquin or ACGIH-recommended threshold limit value for this compound.*

Toxicological Effects: *Monsanto Industrial Chemical Company supported the majority of studies found on chemical disposition and acute, chronic, and reproductive toxicology of ethoxyquin. These studies were conducted by various contract laboratories including*

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[1]The information contained in this Executive Summary of Safety and Toxicity Information (ESSTI) is based on data from current published literature. The summary represents information provided in selected sources and is not claimed to be exhaustive.

Western Utilization Branch, Younger Laboratories, and Hazelton Laboratories.

Human: *No data were found concerning the prechronic, chronic, carcinogenic, reproductive, or teratogenic effects of ethoxyquin in humans. Occupational contact dermatitis has been reported among workers who handle or manufacture animal feed. Positive results to ethoxyquin in skin patch tests occurred in many of the cases reported.*

Animal: *Ethoxyquin was found to be rapidly and almost completely excreted in the urine and feces of rats, cow, and dogs. Highest concentrations of ethoxyquin were generally found in the liver and kidneys of test animals. Accumulation appears to occur in the fat. Identified and unidentified metabolites of ethoxyquin were detected in the urine of test animals. In one study, major metabolites were found to arise primarily from de-ethoxylation of ethoxyquin (40-50%). Residue analysis of muscle and liver tissues and eggs of animals fed ethoxyquin-treated diets demonstrated ethoxyquin levels below tolerances.*

Ethoxyquin has low oral toxicity in test animals. The oral LD_{50} of 20% and 50%, ethoxyquin in rats is 800-1,000mg/kg and the oral LD_{50} for 70% ethoxyquin in rats is 3,300 mg/kg. *The oral LD_{50}s in mice for 0.125% and 0.25% ethoxyquin are 85.7 -94.0 mg/kg and 98.5-112.1 mg/kg, respectively. This compound is a mild skin and eye irritant in test animals. Ethoxyquin was found to significantly decrease body weight, food intake, and occasionally growth rate in rats, cows, dogs, chickens, and mice exposed to prechronic and chronic doses in animal feed. In these studies, ethoxyquin caused a significant increase in liver and kidney weights in test animals. In male rats, adverse effects observed included scars on kidneys, inclusions in hepatic cells, and kidney lesions in the higher dosed groups (500 -4000 ppm). Effects characteristic of chronic glomerulus nephrosis in male rats exposed to 4,000 ppm ethoxyquin were observed. In dogs fed doses of ethoxyquin ranging from 10-100 mg/kg, adverse effects observed included anorexia, abdominal pain, and increased liver and kidney weights. Dose-dependent exogenous pigment was seen in all groups. These animals also exhibited an increased fat content in the collecting tubules of kidneys, suggestive of fatty nephrosis. Limited data were found on the carcinogenic potential of*

ethoxyquin. This chemical has been found to be a weak bladder carcinogen in rats. In mice, ethoxyquin administration caused an increase in solitary adenomas and lymphomas, however, this was not concluded to be indicative of carcinogenicity. A number of studies of the carcinogenic promoting capabilities of ethoxyquin indicated that it enhanced tumor formation induced by other compounds in the forestomach, bladder, glandular stomach, esophagus, distal colon, and kidney in rats. In reproductive studies conducted on rats and rabbits, ethoxyquin was not found to have adverse effects. However, at high doses in chickens, ethoxyquin caused an increased frequency of resorption, and a significant increase in anomalous features in fetuses. A decrease in fertility was seen in ethoxyquin-treated chickens.

Genetic Toxicology: *Numerous mutagenicity tests have been conducted on ethoxyquin in prokaryotic systems. Ethoxyquin was found to be mutagenic to Salmonella typhimurium strains TA98, TA100, and TA1528 in the presence of metabolic activation, but was non-mutagenic in the absence of metabolic activation. In other studies, ethoxyquin was non-mutagenic to Salmonella strains TA1535, TA1537, TA1538, TA98, and TA100 in the presence of metabolic activation. Ethoxyquin was mutagenic to Bacillus subtilis in the rec-assay.*

Structure Activity Relationships: *Ethoxyquin is similar to Quindoxin, a known carcinogen, and Flectol-H, a tumor inducer in rats.*

I. NOMINATION HISTORY AND REVIEW

A. Nomination History

1. Source: Food and Drug Administration [FDA, 1990]

2. Date: January, 1990

3. Recommendations: Carcinogenicity

4. Priority: FDA's fiscal year 1990 priority chemical for NTP carcinogenicity testing

5. Rationale/Remarks:
   - Preservative in animal feed (levels may approach 150 ppm)
   - Herbicide and antidegradation agent for rubber
   - Uncertainty concerning the toxicological effects of ethoxyquin
   - Has induced kidney damage in rats
   - Appears to have a modifying effect on other chemicals (hepatocarcinogens and bladder carcinogens)

B. Chemical Evaluation Committee Review

1. Date:

2. Recommendation:

3. Priority:

4. NTP Chemical Selection Principles:

5. Rationale/Remarks:

C. Board of Scientific Counselors Review

1. Date:

2. Recommendations:

3. Priority:

4. Rationale/Remarks:

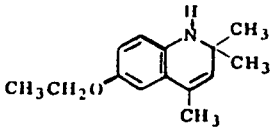
D. Executive Committee Review

1. Date:

2. Decision:

II. CHEMICAL AND PHYSICAL DATA

A. Chemical Identifiers



ETHOXYQUIN

Molecular Formula: $C_{14}H_{19}NO$ Molecular Weight: 217.34

CAS No. 91-53-2
RTECS No. VB8225000

B. Synonyms and Tradenames

Synonyms: 6-ethoxy-1,2,-dihydro-2,2,4-trimethyl; 1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline; 1,2-dihydro-2,2,4-trimethyl-6-ethoxyquinoline; 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline; 6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline; 2,2,4-trimethyl-6-ethoxy-1,2-dihydroquinoline; EMQ; ethoxyquine, EQ

Trade names: Antioxidant EC; Caswell No. 4270; Dawe's Nutrigard; Niflex; Nix-Scald; Nocrac AW; Permanax 103; Quinol ED; Santoflex A; Santoflex AW; Santoquin; Santoquine; Stop-Scald; USAF B-24; Stopscald

C. Chemical and Physical Properties

Description: Brown to brownish-red [Monsanto, 1987], clear [Sax and Lewis, 1987] or yellow [Meister, 1990] liquid; May darken with age [Meister, 1990]; Mercaptan-like odor [Monsanto, 1987].

Melting Point: Approximately 0°C (32°F) [Sax and Lewis, 1987]

Boiling Point: 123°-125°C (253°-257°F) @ 2 mm Hg [Monsanto, 1987]

Specific Gravity:	1.029-1.031 @ 25°C [Monsanto, 1987]
Refractive Index:	1.569-1.572 @ 25°C [Sax and Lewis, 1987]
Solubility in water:	Insoluble [Monsanto, 1959]
Solubility in Other Solvents:	Miscible with animal and vegetable fat and oils [Monsanto, 1959]
Log Octanol/Water Partition Coefficient:	No information found
Reactive Chemical Hazards:	Incompatible with acids. Polymerization may occur at temperatures above 160°C [Monsanto, 1987]. Decomposition products include nitrogen oxides, carbon monoxide and carbon dioxide [Monsanto, 1987].
Flammability Hazards:	• Combustible [Monsanto, 1987] • Vapor Pressure: 1.82 x 10^{-5} mm Hg @ 0° C (purified material); 2.56 x 10^{-4} mm Hg @ 25° C [Monsanto, 1987] • Flash Point: 107°C (225°F) (CC), 140-143°C (285°-290°F) (OC) [Monsanto, 1987]

III. PRODUCTION/USE

A. Production

1. Manufacturing Process

   Ethoxyquin is produced by passing acetone vapor into *p*-phenetidine containing 1% iodine @ 120°-130°C. Ethoxyquin is separated from the reaction mixture by distillation [Budavari, 1989].

2. Producers and Importers

   U.S. Producers:

   - Marshall Thomas Company, Inc.
     Lexington, Kentucky [Meister, 1990]

- Monsanto Agricultural Company
  St. Louis, Missouri [Meister, 1990; USITC, 1989]

- Monsanto Chemical Company
  Nitro, West Virginia [SRI, 1989; USITC, 1989]

- Philipp Brothers Chemicals, Inc.
  New York, New York [Chemical Week Buyer's Guide, 1988]

- Raschig Corporation
  Richmond, Virginia [Chemical Week Buyer's Guide, 1988]

European Producers:

- Bayer AG
  Leverkusen (Nordrhein-Westfalen), Germany [SRI, 1989]

- Chemia S.P.A.
  Dosso (Ferrara), Italy [Meister, 1990]

- Raschig AG
  Ludwigshafen (Rheinland-Pfalz), Germany [SRI, 1989]

- Rexolin Chemicals AB
  Helsingborg (Malmöhus), Sweden [SRI, 1989]

Importers:

- Diamond Shamrock
  Cleveland, Ohio [USEPA, 1990a]

- Kingsley and Keith Chemical Corporation
  Englewood Cliffs, New Jersey [USEPA 1990a]

- Philipp Brothers Chemicals, Inc.
  New York, New York [USEPA, 1990a]

3. Volume

The public file of the EPA TSCA Inventory reported that for the year 1977, 100,000-1,000,000 pounds of ethoxyquin were produced at the following plants: Monsanto Company, St. Louis, MO; Monsanto Company, Putnam, W.V.; and Diamond Shamrock, Cleveland, Ohio [USEPA, 1990a].

In 1981, EPA reported that production of ethoxyquin as an active ingredient had been less than 30,000 pounds within the last five years [USEPA, 1981]. Production data were not reported for ethoxyquin in the United States International Trade Commission's publication, Synthetic Organic Chemicals for the years 1985-1988 [USITC, 1986-1989].

A breakdown of the net quantity of ethoxyquin exported to the United States by country for the years 1985-1988 is presented in Table 1 [U.S. Department of Commerce, 1986-1989]

TABLE 1

Net Quantity of Ethoxyquin Exported to the United States by Country 1985—1988

Source	Countries	Net Quantity (lbs)
U.S. Imports for Consumption* 1985	Belgium	68,519
	Fr. Germany	90,775
	I Israel	79,365
	Israel	361,556
	Japan	139,332
	Other	441
	Total	739,988
U.S. Imports for Consumption 1986	I Israel	950,540
	Israel	36,683
	Japan	145,152
	Total	1,135,375
U.S. Imports for Consumption 1987	Iceland	41,314
	I Israel	990,882
	Israel	66,358
	Japan	423,285
	Total	1,521,839
U.S. Imports for Consumption 1988	I Israel	429,420
	Israel	231,484
	Japan	141,096
	Other	3,929
	Total	805,749

* Imports for consumption is a measure of the total of merchandise that has cleared through Customs, whether such merchandise enters consumption channels immediately, or is withdrawn for consumption from warehouses under Customs custody, or is entered into U.S. Customs territory from Foreign Trade Zones.

4. Technical Product Composition

Ethoxyquin is formulated as an emulsified concentration (EC) containing 52.2% to 70.0% of the active ingredient [USEPA, 1981].

B. Use

- Antioxidant preservative in animal feed [Office of the Federal Register, 1990].
- Antioxidant preservative in select dehydrated forage crops [Office of the Federal Register, 1990].
- Antidegradation agent for rubber production [Office of the Federal Register, 1988].

- Antioxidant used to prevent scald in apples and pears [Office of the Federal Register, 1990].

- Color preservative in paprika, chili powder, and ground chili [Office of the Federal Register, 1990]

IV. EXPOSURE/REGULATORY STATUS

A. Consumer Exposure

The residue levels of ethoxyquin on Bramley's Seedling apples and juice from apples arising from post harvest treatment has been determined by high performance liquid chromatography (HPLC). Apples were obtained from six growers in County Armagh, Northern Ireland between mid-October and mid-November. Apples from growers were dipped in a treatment solution containing Stopscald (70% ethoxyquin) at a concentration of 3 pints per 100 gallon solution. The mean concentration of residues in apples from the six growers were 0.057 mg/kg, 0.116 mg/kg, 0.127 mg/kg, 0.031 mg/kg, 0.010 mg/kg, and 0.239 mg/kg, respectively. Lower residues were found in juices [Hamil and Harper, 1982].

Ethoxyquin residues on apples and pears have not been found to exceed 1.2 ppm in the United States. In New Zealand, up to 2.8 ppm ethoxyquin has been detected on apples. Residues appear to increase with fruit age and elevated temperatures [FAO/WHO, 1970]. The Theoretical Maximum Residue Contribution (TMRC) of ethoxyquin to the human diet from pesticide use is calculated to be 0.12 mg/kg per 1.5 kg diet [USEPA, 1981].

B. Occupational Exposure

Occupational exposure to ethoxyquin may occur among workers manufacturing ethoxyquin, those employed in operations that can release ethoxyquin, agricultural workers, fumigators and pest-control workers, or among transporters of ethoxyquin-containing products. There are approximately 1,000-3,000 workers whose jobs include the application of ethoxyquin to fruit. In addition, an estimated 20 to 90 sorters/wrappers (typically women) used fruit wraps containing ethoxyquin to wrap pears in 1981 [USEPA, 1981].

Data from the National Occupational Exposure Survey (NOES), conducted by the National Institute for Occupational Safety and Health (NIOSH) between 1981 and 1983 indicate that 6,913 male employees were potentially exposed to ethoxyquin in the workplace. No female employees were reportedly exposed [NIOSH, 1990].

C. Environmental Exposure

There are limited data on environmental exposure to ethoxyquin. A report prepared by Monsanto Industrial Chemicals Company indicates that ethoxyquin (Santoflex AW) was detected at levels of 800 ppb in the waste water effluent of a sample plant. In a river die-away test of a nitro plant

discharge, 5 µl of ethoxyquin in a solution with methylene chloride extract was added to 250 ml aliquots of Kanawha River water samples, at approximately 100 ppb. Ethoxyquin was not detected in either active or sterilized water after day three of the experiment, suggesting that this compound may be chemically unstable [Monsanto, 1978].

D. Regulatory Status

- There is no OSHA permissible exposure limit (PEL) for ethoxyquin.
- Ethoxyquin is regulated by the Food and Drug Administration in 21 CFR parts 172.140, 177.2600 (iii), 573.380, and 573.400 and 40 CFR part 180.178 [Office of the Federal Register, 1988, 1989, 1990].
- The maximum quantity of ethoxyquin permitted to be used in dehydrated forage crops and treated poultry feed is 150 ppm [Office of the Federal Register, 1990].
- The FDA has established a tolerance of 0.5 ppm for residues of ethoxyquin in or on the uncooked meat and meat by-product (including milk and eggs) and 5.0 ppm for cooked meat and meat by-products of animals fed forage crops or feed supplements treated with ethoxyquin. For uncooked liver and fat of poultry fed treated feed, a tolerance of 3.0 ppm has been established. Tolerance residues of 3.0 ppm have been set for apples and pears [Office of the Federal Register, 1989, 1990].
- Ethoxyquin may be safely used as an antioxidant for preservation of color in the production of chili powder, paprika, and ground chili at levels not in excess of 100 ppm [Office of the Federal Register, 1990].
- The World Health Organization has established an acceptable daily intake of 0.06 mg/kg of ethoxyquin for humans [FAO/WHO, 1977].
- New Zealand, the United Kingdom and Canada have established a tolerance level of 3.0 ppm for ethoxyquin on apples. Canadian tolerance levels of 3.0 ppm for ethoxyquin in or on livers of poultry, and 0.5 ppm for ethoxyquin in or on meat, poultry and eggs have also been established [FAO/WHO, 1970].

E. Exposure Recommendations

- There is neither an ACGIH-recommended threshold limit value-time weighted average (TLV-TWA), nor a NIOSH-recommended exposure limit (REL) for ethoxyquin.

V. TOXICOLOGICAL EFFECTS

A. Chemical Disposition

1. Human Data

No data were found in the literature on chemical disposition of ethoxyquin in humans.

2. Animal Data

Numerous studies on the chemical disposition of ethoxyquin in animals have been conducted.

- In an acute, single-dose study, rats (number and strain not specified) were fed 0.005% unlabeled ethoxyquin (50 ppm) in the diet for 10 days, and then fed a single dose of 1.5 mg of carbon-14 labeled ethoxyquin by stomach tube. Respiratory, urine, and feces samples were collected for analysis. Females were sacrificed after 1, 2, and 7 days, and males were sacrificed after 7, 14, and 28 days. Tissue analysis indicated that the liver and kidney contained the highest concentrations (0.3 ppm - 0.4 ppm) of radioactivity after one week. Other tissues which contained lower levels of radioactivity included the heart, skeletal muscle, and brain. The spleen, blood, and abdominal fat contained intermediate levels of radioactivity. After one week, 0.09 ppm labeled ethoxyquin was found in the muscle, and after two weeks the level dropped to nearly zero. After one to four weeks the ethoxyquin level in fat dropped from 0.2 to 0.1 ppm. The estimated ethoxyquin turnover rate in rats was 10 days. Measurements of the percentage of ^{14}C-ethoxyquin excreted in the urine and feces indicate that ethoxyquin is rapidly excreted within one or two days. Oxidation of ethoxyquin to carbon dioxide was negligible [Wilson et al., 1981].

- In a similar study, a group of rats were fed a diet containing 0.005% ethoxyquin for 10 days, and then fed a diet containing 0.005% carbon-14 labeled ethoxyquin for 10 days. The highest concentrations of ethoxyquin found in the liver and kidney were 2.1 ppm and 4.8 ppm, respectively in 2 males and 2 females. The spleen, muscle, and fat contained 1.0 ppm, 0.3 ppm, and 0.8 ppm of ethoxyquin, respectively.

  In the same study, two animals which had become pregnant (unknown day) were immediately fed the labeled diet. Both animals delivered nine days later. Placental transfer of ethoxyquin or a metabolite reportedly occurred. The newly born pups' stomach contents contained 0.15 ppm and 0.21 ppm of carbon-14 labeled ethoxyquin respectively. Pups which were sacrificed after one day of feeding contained 0.12 ppm and 0.2 ppm ethoxyquin, respectively. The author suggested that these findings support ethoxyquin transfer *in utero*. The concentration of ethoxyquin, or its metabolites, in coagulated rat's milk was 0.19 and 0.12 ppm [Wilson et al., 1981].

- In an acute oral feeding study, two cows were conditioned with a 0.015%

ethoxyquin diet, then fed a 1575 gram mixture of alfalfa meal with 17.5% molasses and 1.0% cottonseed oil, containing 155 milligrams ^{14}C-ethoxyquin. Urine, feces, milk and respired air were obtained for the next 3.5 days. Measurements of ^{14}C-ethoxyquin* in the feces and urine were 45.3 mg and 107.9 mg, respectively, indicating rapid and complete excretion of ethoxyquin. Seven milk samples were taken from the cows daily. The highest concentration of ^{14}C-ethoxyquin* (0.036 ppm), was found in the third milk sample taken 33 hours after dosing. The labeled ethoxyquin was detected in the skim section of cow milk [Wilson et al., 1981].

- Three Holstein cows which ate at least 15 kilograms of dry matter per day were fed 1.125 grams of ethoxyquin in a concentrate at each milking for 13 days. Evening milk samples were taken three times weekly for one week before the supplementation, during the supplementation and for 5 days after supplementation. Ethoxyquin levels in milk were determined. Low levels of both ethoxyquin and an unknown compound were identified. The maximum concentration of ethoxyquin in milk was found to be less than 7 µg/liter [Dunkley et al., 1968].

- Albino, adult, male rats were given a single dose of 104 mg/kg ^{14}C-ethoxyquin in arachis oil by oral intubation. Two rats were sacrificed at each of the following times after administration: 0.5, 1, 2, 4, 8, 10, 12, 16, 20, 24, 48, and 144 hours. Autoradiograms prepared at 0.5 hours after administration of ethoxyquin indicated that radioactivity was distributed throughout the blood and tissues. Throughout the experimental period, the highest radioactivity was detected in the liver, kidney, gastrointestinal tract, and adipose tissue. At 0.5 hours and 6 days following dosing, concentrations in the liver were found to be 2.2% and 0.2%, respectively, with the highest level detected 8 hours post-sacrifice. After 6 days, 7.5% of this peak hepatic level was retained. At 48 hours, the main sites of retention (apart from the liver) were the intestines, renal medulla, and blood. By day 6, radioactivity in the renal medulla had declined, but remained high in the renal cortex. Residues of ethoxyquin and metabolites were also present in the intestine, lung and various adipose tissues. The authors suggest that the high and persisting radioactivity in the liver indicates that ethoxyquin metabolism may occur in this organ. Also, the slow excretion of ethoxyquin may be due to its accumulation in adipose tissue and slow release from this tissue [Skaare and Nafstad, 1979].

- Fifteen male rats were dosed directly into the stomach with 40 milligrams of labeled ethoxyquin. Groups of 3 rats were sacrificed at 6, 12, 24, 48, and 72 hours after administration of the compound. Radioactivity in the organs, urine and feces was determined. The highest values of the administered dose observed in the liver, kidney, and stomach were 8.0%, 0.3%, and 56.5%, respectively, after 6 hours. Levels of radioactivity found in the small intestine, large intestine, caecum and blood were

* Measured as radioactivity

11.3%, 0.9%, 6.9% and 0.9% of the administered dose, respectively. The authors suggest that the intensive absorption of ethoxyquin in the small intestine is due to continuous recirculation of carbon-14 activity. After 2 days, carbon-14 labeled ethoxyquin levels were found to fall drastically in organ fractions and to rise in urine and feces. Labeled ethoxyquin concentration in urine exceeded that in feces by two-fold. After 72 hours, 84.8% of the total labeled dose was found to be eliminated in the urine and feces. Unchanged ethoxyquin, one unknown metabolite and five known metabolites were detected in urine including N-acetyl-6-acetoxy-1,2-dihydro-2,2,4-trimethylquinoline; monohydroxyethoxyquin; 6-ethoxy-1,2,3,4-tetrahydro-2,2,4-trimethylquinoline; 6-hydroxy-1,2-dihydro-2,2,4-trimethylquinoline dihydroxyethoxyquin [ter Meulen, et al., 1980].

- In a chronic feeding study, male and female rats (5-10 per dose level) were fed carbon-14 labeled ethoxyquin for 200, 430, and 700 days in the diet at 0, 0.0062, 0.0125, 0.025, 0.05, 0.1, 0.2, and 0.4%. Rats dosed with 0.2 and 0.4% labeled ethoxyquin had some ^{14}C-ethoxyquin accumulation in fat (amount not specified). Paper chromatographic analysis of acetone extracts of the fat, liver, and kidneys from animals receiving 0.4% labeled ethoxyquin detected no radioactivity. Urinalysis of excretes revealed minimal unchanged ethoxyquin. In rats receiving 0.4% labeled ethoxyquin in diet, 0.32-0.52% ^{14}C-ethoxyquin appeared unchanged in the urine [Western Utilization Research Branch, 1955].

- A metabolism study in which mongrel dogs were fed 50 mg/kg (capsule form) of ethoxyquin indicated that four distinct, metabolites appeared in urine. These metabolites were not identified. An increase in aromatic and residual phenols was noted in the dog urine. The authors suggest that the oxidation of ethoxyquin may lead to the formation of compounds containing phenolic groups. Test dogs had increased excretion of alkoxyl groups compared to controls, but this increase was not proportional to the dosage of ethoxyquin administered [Hazelton Laboratories, 1955].

- In a study to determine the metabolites of ethoxyquin, male albino rats were administered a dose of ethoxyquin as a suspension in soya oil (1 ml) by stomach tube. Doses employed were 100 and 400 mg/kg unlabeled ethoxyquin, or 100 mg/kg ^{14}C-ethoxyquin. After 24 hours, 67-80% of the administered dose of the radioactive compound was recovered in the urine and feces in male rats. Less than 0.1% of the administered radioactivity was detected in 24-hour respired air samples. Approximately 95% of the dose was excreted within 6 days. The major metabolic reaction was de-ethylation to yield 6-hydroxy-2,2,4-trimethyl-1,2-dihydroquinoline, and an oxidation product, 2,2,4-trimethyl-6-quinolone. Other metabolic reactions included hydroxylation to give four different hydroxylated metabolites and one dihydroxylated metabolite. The products were identified by gas chromatographic/mass spectrometric analyses [Skaare and Solheim, 1979].

- In a metabolic study of the excretory pattern of ethoxyquin, a female dog weighing 3,950 grams was given 80 grams of Ken-L-Ration containing 17.40 milligrams of ^{14}C-ethoxyquin. Four hours after dosing, the dog was

sacrificed. Paper chromatographic analysis of the urine, and acetone extracts of liver and kidney samples, demonstrated that ethoxyquin was metabolized in the dog to a compound of a more acidic nature. The authors note that two quinoline derivatives, xanthurenic acid and kynurenic acid, are acidic and readily excreted.

In this same study, another dog was fed 100 milliliters of a 50/50 egg yolk/milk mixture containing 50 milligrams of ^{14}C-ethoxyquin. Urine was collected for 24 hours. The urine was adjusted to pH 8-10 and extracted with chloroform. Five percent of the administered dose was recovered from the chloroform extracts. However, following acid hydrolysis of the chloroform extracts, 84% of the dose was recovered. The authors suggested that ethoxyquin was excreted in some form of an easily hydrolyzed conjugate involving the imino group [Monsanto, reference 19, date not specified].

- Dog (strain not specified) urine samples from control animals and animals fed ethoxyquin at a concentration of 50 mg/kg/day has been analyzed (for unspecified days). Urine from treated animals was darker than controls. An estimate of ethoxyquin excretion in the urine was 1.65-3.15 mg/day or 0.32% and 0.62% of the administered dose. However, ethoxyquin was not detected in concentrated extracts of dog urine, indicating that at least 16,000 times as much ethoxyquin was ingested as was excreted in the urine. No other data was provided [Western Utilization Research Branch March, 1955].

- In a study on the biliary excretion and intestinal absorption of ethoxyquin, male albino rats were divided into 3 groups and surgically prepared with bile duct or abdominal thoracic duct canals. ^{14}C-ethoxyquin was administered to groups 1 and 2 through infusion catheter at 100 mg/kg in arachis oil. Group 3 received 100 mg/kg carbon-14 labeled ethoxyquin in arachis oil by stomach tube. Bile or lymph was collected at hourly intervals for 12 hours, and then for 12- and 24-hour intervals. In groups 1, 2, and 3, 26%-33%, 33%-42%, and 9%-30%, of the administered radioactivity respectively, was excreted in bile 12-24 hours after dosing. Approximately 3% of labeled ethoxyquin was absorbed by intestinal lymphatic absorption. Gas chromatographic/mass spectrometric analyses revealed that 75 to 85% of the radioactive material excreted in the bile was unchanged ethoxyquin. The following metabolites were isolated and identified: 8-hydroxy-ethoxyquin; hydroxylated 8-hydroxy-ethoxyquin; 6-ethoxy-2,2,4-trimethyl-8-quinolone; hydroxylated 6-ethoxy-2,2,4-trimethyl-8-quinolone; 6-ethoxy-2,4-dimethylquinoline, and 2,2,4-trimethyl-6-quinolone [Skaare, 1979].

- In a metabolic study of dogs, 7 male and 5 female (1-2 males and 1-2 females per group) mongrel dogs were divided into 4 groups and received the following dosing regimen of ethoxyquin: control group 1, no test compound; group 2, 10 mg/kg; group 3, 50 mg/kg; and group 4, 100 mg/kg. The test compound was administered orally in capsule form once per day, five days per week. Group 4 was discontinued due to toxicity and group 5 was started and received 3.0 mg/kg ethoxyquin. Four

compounds were detected in the urine of test dogs from group 3; however, no ethoxyquin was detected. The authors concluded that ethoxyquin was not eliminated in the urine. Groups receiving ethoxyquin at 3 mg/kg and 10 mg/kg had glucuronate levels of 1.4 mg and 1.6 mg in 24-hour samples. The control dogs excreted 3.3 mg and 5.4 mg glucoronates. An increase in aromatic and residual phenols occurred in the urine of the dogs fed ethoxyquin [Hazelton Laboratories, 1955].

In an addendum to the final report on this study, it was reported that dogs receiving 3.0 mg/kg/day excreted an average of 40.4 μg and 9.3 μg of ethoxyquin, in the urine and feces respectively, after 24 hours. Dogs receiving 10 mg/kg/day excreted an average of 511.0 μg in urine and 143.4 μg ethoxyquin in the feces after 24 hours. Two dogs in the 50 mg/kg/day group excreted 144.3 μg and 36.2 μg ethoxyquin, in urine and feces respectively, after 24 hours. Based on these results the authors conclude that ethoxyquin is excreted primarily from the kidneys [Hazelton Laboratories, 1955].

- Two lambs, two pigs, and two calves within one month of weaning were fed 30 ppm of carbon-14 labeled ethoxyquin mixed with starter ration for 10 days two times a day. On day 11, animals were sacrificed. No detectable labeled ethoxyquin was found in edible muscle. However, 0.14 ppm-0.28 ppm ^{14}C-ethoxyquin was found in the pig and lamb liver [Monsanto, date not specified].

- A study was conducted to determine residues of ethoxyquin in poultry tissues and eggs. Twelve Shaver hens, and twenty Cobb 1-day-old broiler chicks (in two groups of ten males and ten females) were administered a feed containing 125 mg ethoxyquin/kg feed, twice weekly. Eggs were collected over a period of 4 weeks from the Shaver hens. Commercial eggs served as controls. The average ethoxyquin concentration in whole eggs was 0.031 mg/kg. Levels of ethoxyquin in all tissues except liver and body fat were less than 0.005 mg/kg. Broiler male liver ethoxyquin levels were 0.063 mg/kg and Shaver hen liver levels were 0.048 mg/kg. The highest tissue concentrations were found in the loose abdominal fat of all birds. Test birds had 0.19-0.25 mg/kg ethoxyquin in fat, whereas commercial broilers had 0.015 - 0.051 mg/kg ethoxyquin in their fat [Hobson-Frohock, 1982].

- To determine the minimum dietary level of ethoxyquin which produces no more than 1.0 ppm residue in dogs, mongrel puppies were fed 5-10 grams of food containing 10-100 ppm of ethoxyquin and a mixture of egg yolk/milk from hens that had received 0.075% ^{14}C-ethoxyquin (for at least 20 days). Dogs were fed on the following regimen for 4 days: meat (morning), 80-100 milliliters yolk milk mixture (noon), and a small amount of meat (night). Overnight they were given 100 milliliters of milk and 100 grams of food. Dogs were sacrificed 15-17 hours after their last meal. It was assumed that for dietary dosage levels less than 2 ppm, the amount of residue detected in dog liver would be 0.1 ppm or less. No reactivity was detected in the dog liver. The authors concluded that a dog can consume 0.3-0.4 milligrams ethoxyquin/kg body weight indefinitely

without accumulating greater than 0.1 ppm of this compound in the liver. This data was used to calculate the ratio of human intake dosages of ethoxyquin that would produce residues in excess of 0.1 ppm in the liver.

The authors assumed the daily consumption by infants of a poached egg yolk would represent the greatest chance for possible human exposure to ethoxyquin. It was calculated that human infants consuming a lightly poached yolk every day would receive less than 1/236 of the average dose administered to puppies, and less than 1/1,574 of the maximum tolerated chronic dog dose observed in the Hazelton Laboratories' study described above [Monsanto, date not specified].

B. Acute

1. Human Data

Ethoxyquin has been associated with contact dermatitis in workers handling animal feeds and fruits treated with ethoxyquin. Generally, the dermatitis is characterized by an itchy, erythematous reaction that appears to be related to air-borne exposures. In addition, there is some evidence of sensitization among workers who have experienced contact dermatitis due to ethoxyquin exposure. No other data were found on acute effects associated with ethoxyquin exposure in humans.

2. Case Reports

The following cases represent data on the dermatological effects of ethoxyquin:

- A 56-year-old farm owner who used a feed containing ethoxyquin reported having an itchy, erythematous, scaly dermatitis with occasional oozing eruptions. The dermatitis started on his face and continued to his hands and forearms. Because the disorder resembled a photodermatitis, a light sensitivity reaction was suspected. Patch testing was positive for ethoxyquin at 1.0% and 0.2%. The dermatitis cleared after removal from the source exposure [Van Hecke, 1977].

- A 44-year-old male worker who was employed at a factory producing animal feeds had symptoms including erthymatous, slightly scaly eruption on his face, hands, arms and legs. This worker tested positive for ethoxyquin at concentrations of 1.0% and 0.2%. The dermatitis cleared after removal from the source of exposure [Van Hecke, 1977].

- A 21-year-old female student whose family bred poultry developed a pruritic erythematous vesicular dermatitis near her mouth and on the backs and sides of the fingers of her right hand. The dermatitis spread to the fingers of her left hand. The student helped to vaccinate chickens 2 to 3 times per month. Patch testing with 0.5% ethoxyquin revealed positive reactions at 48 and 72 hours. The chicken feed used by the family was later found to contain ethoxyquin [Savini, et al., 1989].

- A 52-year-old worker in the feed-mixing department of a grain firm developed nearly universal exfoliative dermatitis after 10 days of exposure. He demonstrated a strong positive reaction to 0.5% ethoxyquin. After treatment, he was moved to another department in the firm, but his dermatitis reappeared, localized to the face and hands. The dermatitis persisted even after his departure from the workplace. In this case, ethoxyquin also appeared to elicit photosensitivity, as the worker reacted to a range of ethoxyquin concentrations (0.50-0.01%), both irradiated and non-irradiated [Zachariae, 1978].

- A 53-year-old animal feed mill worker developed dermatitis and tested positive for ethoxyquin at 0.5% in alcohol. His lesions cleared when he was moved to another work place [Brandao, 1983].

- In an abstract from a foreign study, it was stated that acute contact dermatitis among feed workers was due to ethoxyquin in feedstuffs. The sensitization potential of ethoxyquin was characterized as strong [Schubert et al., 1973].

- Two other cases concerning dermatitis among animal feed mill workers have been described. Both workers had positive reactions to patch testing with 0.5% ethoxyquin. In one worker, the rash began on the palms and spread to all parts of both hands and feet. Removal from exposure cleared both cases [Burrows, 1975].

- Twenty-five cases of ethoxyquin-induced dermatitis among apple workers in British Columbia were reported. The workers were exposed to ethoxyquin at 0.3% in solution which had been added to the apples in storage. No other data were reported [Wood and Fulton, 1975 as cited in Burrows, 1975].

3. Animal Data

- As described in the USEPA Pesticide Registration Standard for ethoxyquin, oral LD_{50}'s of 3,150 mg/kg for rats and 3,000 mg/kg for mice have been reported in a Russian study. No other information was provided [USEPA, 1981].

- The acute oral LD_{50} for 70% ethoxyquin in rats (strain, sex, and number not specified) was reported to be 3.3 g/kg in an unpublished study conducted by Younger Laboratories in 1960. Symptoms observed included diarrhea, lethargy, weight loss, and collapse. Upon autopsy, inflammation of gastric mucosa and renal congestion were observed [USEPA, 1981].

- In rats (strain and sex not specified), 20% and 50% solutions of ethoxyquin in cottonseed oil were administered by stomach tube at doses ranging from 125 to 1000 mg/kg. Five of 7 rats receiving 1000 mg/kg, 2 of 5 rats receiving 800 mg/kg, and 1 of 5 rats receiving 640 mg/kg died. Depression was seen in rats just before death. The oral LD_{50} in rats was calculated to be 800-1,000 mg/kg [Western Utilization Research Branch,

1954].

- Mice (strain and sex not specified) were administered ethoxyquin mixed with tween 80 and saline (2-5% emulsions) via intraperitoneal and intravenous routes. Mice were injected (n=1-7) intraperitoneally with 125, 250, 400, 500, 625, 800 and 1000 mg/kg ethoxyquin, and intravenously with 100-400 mg/kg ethoxyquin. Animals in the 1000 mg/kg group became comatose within minutes, and 5 of 7 mice died within 1 to 2 days. No animals died at doses of 800 mg/kg or less. Intravenous injection of lethal doses caused convulsions and jumping followed by prostration and coma. Death occurred within 2 minutes to 2 hours. Both intraperitoneal and intravenous injections of toxic doses of ethoxyquin caused depression. Symptoms observed in all toxified animals included excitement, uncertain gait, and prostration. The intravenous LD_{50} in mice was to determined be 178 mg/kg [Western Utilization Research Branch, 1954].

- Male and female marmosets unexpectedly died within 2 days after being fed a new batch diet. Two of the surviving marmosets were sacrificed. A significant (p value not specified) decrease in body weight in all marmosets fed the diet was seen. Ethoxyquin was identified in the food at concentrations ranging from 652 ppm-846 ppm. Observed acute effects included minimal gross lesions, evidence of liver and kidney damage, an empty gastrointestinal tract, a distended gall bladder and hemolytic anemia as well as muscle wasting, depression, a tarry odor and dark copper colored livers [McIntosh et al., 1986].

- To determine the toxic level of ethoxyquin in lab chow, 3 groups of hooded Wistar rats were fed the following test diets: Group A, low ethoxyquin, (80 ppm); Group B, toxic dose ethoxyquin, (652 ppm); and Group C, normal rat chow. A decrease in growth and food intake was seen in group B. Pathological examination of the rats post-sacrifice revealed no abnormalities.

  One month later, deaths among breeding female rats and fetal stunting were observed in the lab rat colony. Food was analyzed and 715 ppm ethoxyquin was detected. The authors concluded these observations suggest that a ten fold addition of ethoxyquin in chow over recommended concentrations produced significant mortality in common marmosets, and decreased growth in breeding rats [McIntosh et al., 1986].

- In an inhalation study, 15 Sprague Dawley albino rats (male and female) were exposed to 1750 ppm ethoxyquin for 6 hours. No adverse effects were observed after 14 days [Scientific Associates, 1960].

- Skin absorption studies were conducted on New Zealand white rabbits (6 male and 3 female) at doses ranging from 5,000 to 12,600 mg/kg of 70% ethoxyquin. No symptoms of ethoxyquin-induced systemic toxicity were observed. However, lethargy and weight loss were noted [Younger Laboratories, 1960].

- A Draize skin sensitization test using a 70% active ethoxyquin emulsion conducted on 3 albino rabbits for up to 24 hours produced trace erythema, demonstrating that ethoxyquin is a mild skin irritant [Younger Laboratories, 1960].

- In an eye irritation test using undiluted 70% ethoxyquin emulsion on 3 albino rabbits, ethoxyquin was classified as a "mild eye irritant". Adverse effects produced upon application included blinking and mild discomfort [Younger Laboratories, 1960].

- The dermal rabbit (strain not specified) LD_{50} was reported to be 3160 mg/kg-5010 mg/kg. This source also reports that ethoxyquin is a slight eye and mild skin irritant in rabbits [Monsanto, 1983].

- The following series of acute toxicity tests, supported by Monsanto, were run on White Leghorn cockerels:

  In trial I, 55 nine-week-old cockerels were divided into 11 groups, 5 birds per group. The cockerels were kept off feed for 12 hours prior to oral administration of 0.5, 1.0, 2.0, 4.0, and 6.0 g/kg of ethoxyquin, in oil form. The 0.5 and 1.0 g/kg group cockerels became temporarily ill; effects observed included huddling together, incoordination, and anorexia for the first 2 hours. The 2.0 g/kg group demonstrated more pronounced effects and death occurred in one bird within 72 hours. In the 4.0 and 6.0 g/kg group, a 40% mortality rate was observed at 4-5 days, and 36 hours, respectively. No effects were observed in controls.

  In trial II, 11 eight-week-old cockerels were divided into 5 groups, 2 birds per group, and kept off feed for 12 hours. Ethoxyquin in propylene glycol was administered by stomach tube at 6.0 g/kg. A 40% mortality rate was observed at 36 hours. Symptoms similar to these described for trial I were observed.

  In trial III, twelve-week old cockerels were divided into 6 groups, 5 birds per group, with one control bird. Ethoxyquin was administered in propylene glycol at 6.0, 8.0, and 10.0 mg/kg. A 60% mortality was observed in birds at the 6.0-8.0 g/kg dose levels within 24 hours. Symptoms similar to those described for trial I and II were observed.

  In trial VI, 128, eight-week-old cockerels were divided into 20 groups, 6 per group, and 2 groups (4 per group) for control birds. On- and off- feed trials were conducted at 2.0, 4.0, 6.0, 8.0, and 10.0 g/kg. Controls were given mazola oil. An MLD_{50} of 10 g/kg was determined for birds kept off-feed and 8.0-10.0 g/kg for birds on-feed. Gross observations included emaciation, enlarged gall bladder, hemorrhagic enteritis, and loose green fecal matter, and ethoxyquin present throughout the gastrointestinal tract [Western Utilization Branch, 1954].

C. Prechronic

1. Human Data

No data were found in the literature concerning the prechronic effects of ethoxyquin on humans.

2. Animal Data

- In a skin irritation study, a single drop of undiluted ethoxyquin was rubbed over a 2 centimeter diameter section of rabbit and guinea pig skin (strain and number of animals not specified). Daily applications (except on weekends) were continued for 2 weeks. Slight erythema, followed by papular eruption, and in some cases scab formation was observed. Symptoms gradually cleared after discontinuation of treatment. Within 2 weeks, the skin appeared normal.

  Also, equal amounts of ethoxyquin and tween 80 did not cause sensitization in injected guinea pigs and rabbits. No other data were provided [Western Utilization Research Branch, 1954].

- Twenty-five female ICR mice were divided into 5 groups, 5 mice per group. Four test groups received powdered feed containing 0.25 or 0.5% ethoxyquin or ethoxyquin-hydrochloride. The control group was given feed without the test compound. After 20 days of treatment, mice were sacrificed and tissues were prepared and analyzed. Mice fed ethoxyquin or ethoxyquin-hydrochloride experienced a dose-dependent weight loss, decreasing with time. Dose-dependent hepatic hypertrophy among ethoxyquin and ethoxyquin-hydrochloride treated mice was significant ($p<0.0001$) compared to controls. Liver weights in the high dose group (0.05%) were greater in the ethoxyquin group than those in the ethoxyquin-hydrochloride group. Tissue thiol levels increased in both ethoxyquin and ethoxyquin-hydrochloride groups, especially in the small intestine ($p=0.007$). Liver tissue thiol levels also increased significantly ($p=0.116$). Thiol levels in the stomach of ethoxyquin treated rats were significantly higher ($p=0.024$) than levels observed in the ethoxyquin-hydrochloride and control groups [Kim, 1985].

- In a 6-week study, male F344/DuCrj rats were administered 5,000 ppm ethoxyquin in their diet daily. Ethoxyquin was not found to induce toxic hepatic lesions. Ethoxyquin did induce toxic proliferative biliary lesions, but not oval cell hyperplasia or cholangiofibrosis [Ward et al., 1989].

- SCWL chicks were fed the following test diet: group 1 (230 chicks) received 5% alfalfa; group 2 (221 chicks) received 5% alfalfa combined with ethoxyquin; group 3 (223 chicks) received 5% alfalfa combined with 0.15% ethoxyquin; and group 4 (227 chicks) received 5% alfalfa combined with 1.5% ethoxyquin for 10 weeks. Final ethoxyquin doses were 0, 0.00075%, 0.0075%, and 0.075%. Viability and growth were the same for all groups during the 10-week period.

On day 383, the hens were autopsied. Upon micropathological examination, spotty discoloration of livers was seen in treated and control hens. Because these effects were also seen in controls, the authors concluded they were not due to ethoxyquin treatment.

Cockerels were autopsied on day 450. An increase (p value not reported) in testes weight was observed in test animals in the test groups exposed to 0.15% and 1.5% ethoxyquin. Liver and kidneys had focal leucocytic infiltrations in both control and test groups [Western Utilization research Branch, 1954].

- Four-day-old SCWL cockerels, were divided into 6 groups of 70 chickens each. Chickens were fed starter diet fortified with megasol for 12 weeks. The 5% alfalfa diet was dosed with the following concentrations of ethoxyquin: 0, 0.00075%, 0.0015%, 0.0030%, 0.0075%, and 0.075% ethoxyquin. No adverse effects on body weight, feed consumption, livability and micro- and macro-anatomy were seen in any test groups. These results were obtained from a study of the effect of ethoxyquin on reproduction (see Section V.E) [Western Utilization Research Branch, 1954].

  The no-effect level (NOEL) for microscopic pathological changes in this study was determined to be 28 mg/kg/day [Monsanto, 1983].

- Three groups of pigs, six per group, were fed ethoxyquin at 0 (control group), 10 times and 100 times respectively, the amount of the chemical which they would expect to have received from a ration containing 10% alfalfa treated with 0.015% ethoxyquin. No adverse effects were observed [Western Utilization Research Branch (University of Illinois), 1954].

- Four male calves from the University of Illinois dairy herd were fed 6.8 grams of ethoxyquin and 2 were fed 0.68 grams ethoxyquin daily in alfalfa feed. No adverse effects were observed in the lower dosed group, however, all animals died in the high dose group. The authors reported that death may have been caused by starvation since there was difficulty in getting the high dose group to eat [Western Utilization Research Branch (University of Illinois), 1954].

- Three groups of male albino rats were administered the following doses: 0 mg ethoxyquin (control group 1); 150 mg ethoxyquin per liter drinking water (group 2); and a single oral dose of 500 mg ethoxyquin/kg via stomach tube (group 3). All groups received pelleted rat chow without ethoxyquin for 2 months. Groups 2 and 3 had increased liver weights (23% and 38%, respectively) compared to group 1. In addition, liver protein and cytochrome p450 were decreased in groups 2 and 3. Ethoxyquin measured 6.5-8.5 times higher in the liver and plasma of group 3 compared to group 2. Ultrastructural changes were observed in group 3 only. Proliferation of the smooth endoplasmic reticulum occurred in the parenchymal cells. Small changes in the mitochondria were also observed. The most severe damage observed involved alterations in the

lining cells. Group 2 showed slight proliferation of the endoplasmic reticulum only [Nafstad and Skaare, 1978].

D. Chronic/Carcinogenicity

1. Case Reports

- A 66-year-old worker at a pig feed firm developed a universal dermatitis after 20 years of work. This worker tested positive for ethoxyquin. In addition, this worker had experienced multiple basal cell carcinomas on numerous occasions. In his report, the author noted that this finding may be of particular interest, since ethoxyquin has "certain similarities" to quindoxin, a "known" carcinogen [Zachariae, 1978].

No other data were found in the literature concerning the chronic effects/carcinogenicity of ethoxyquin in humans.

2. Animal Data

- In a chronic oral feeding study, 2 trial experiments were conducted on weanling albino rats.

In trial 1, 10 male and 10 female rats were continually fed a diet containing 0.0, 0.2, 0.4% ethoxyquin in alfalfa. On day 155, males and females in the 0.4% dose group weighed significantly less ($p<0.05$ and $p<0.01$, respectively) than control rats. Rats in the 0.2% group also weighed less (not significant) than controls.

In trial 2, groups of 10 male rats were placed on diets containing 0, 0.0062, 0.0125, 0.025, 0.05, 0.1, and 0.2% ethoxyquin and 10 females were fed diets containing 0, 0.05, 0.1, and 0.2% ethoxyquin for 225 days. In addition, 5 females were fed diets containing 0.0125 and 0.025% ethoxyquin. Half of the male animals in the 0, 0.2, 0.1., 0.05 and 0.025% ethoxyquin and half the females in the 0, 0.2, 0.1% dose groups were sacrificed on day 225. A small indication of kidney lesions was seen in the 0.1 and 0.2% ethoxyquin groups. Liver weights among females in the 0.2% ethoxyquin group and males in the 0.1% group as well as liver weights in all higher dose groups were significantly greater ($p<0.01$) than controls. Kidneys weights were significantly greater ($p<0.01$) in the 0.2% (trials 1 and 2) and 0.4% (trial 1) dose groups versus controls. Upon visual observation of kidneys of 2 male rats in the 0.4% ethoxyquin group, stones in the renal pelvis were noted. Microscopic examination revealed well-developed chronic pyelonephritis among males in the high dose groups. Two kidneys had areas of calcification. The thyroid glands of male rats exposed to higher doses of ethoxyquin had evidence of hyperplasia. All male rats in the 0.2% ethoxyquin group, 3/4 in the 0.1% group, and 2/5 in the 0.05% group had small kidney scars [Western Utilization Research Branch, 1954].

- As a supplement to the above study, the surviving rats (5-10 males and 5-10 females) were fed ethoxyquin in their diet at the same concentrations

as the male rat diet described above. Mortality occurred at the 700th day due to infection. Pathological inspection revealed a pitted surface of the kidneys in male rats at the 0.2% and 0.1% ethoxyquin dose groups. The authors state that other observed gross changes bore no apparent relationship to ethoxyquin including respiratory tract infection, tumors, cystic ovaries, and infected uteri [Western Utilization Research Branch, 1955].

In this study, the NOEL for microscopic pathological changes was calculated to be 13 and 15 mg/kg/day for male and female rats respectively, at day 225 [Monsanto, 1983].

- In a one year chronic oral administration study supported by Monsanto, 7 male and 5 female mongrel dogs were divided into 4 groups (mixed male and female) and put on the following dose regimen: no test compound (group 1; 1 male, 1 female); 10 mg/kg ethoxyquin (group 2; 2 males, 1 female); 50 mg/kg ethoxyquin (group 3; 1 male, 2 females); and 100 mg/kg ethoxyquin (group 4; 1 male, 2 females). All doses were administered orally in capsule form, five days per week, with the exception of group 3, which received one dose per day for the first 6 weeks, and 2 doses per day thereafter. Group 4 administration was discontinued after 6 weeks due to toxicity. Group 4 dogs were sacrificed at week 9 and replaced by group 5 (1 male, 1 female), which received 3 mg/kg ethoxyquin. Gross examination revealed abdominal tenderness in one dog in groups 2, 3, and 5, within 5 weeks. Anorexia occurred in 2 dogs in group 4 and 1 dog in group 5. Depression and soft feces were also observed in one group 5 dog. Dark brown livers were seen in groups 2 (associated with evidence of intestinal parasitic infestation), 3, and 4. Urine was also discolored in groups 3 and 4, with occasional appearance of globules. Group 3 dogs developed irritation of the large and small intestine. One dog in group 5 had an enlarged liver. Bromosulphalein liver function was decreased in groups 2, 3, and 4 as early as the seventh week. All dogs in group 4 and one in group 3 had erythrocyte sedimentation. Upon microscopic examination, liver and kidney changes were seen in all groups. Kidney swelling with fatty accumulation was seen in groups 2, 3, and 5. Granulation of cells in collecting tubules was also seen in groups 2 and 3. Liver degeneration and fatty metamorphosis of the liver was seen in groups 2, 3, and 5. Groups 3 and 4 exhibited marked retention of exogenous pigment in the liver. One dog in group 3 had massive fibrosis. However, there was no evidence of permanent damage or destruction of tissue in the test animals in which liver and kidney changes were evident, suggesting that the histological changes observed may be reversible [Hazelton Laboratories, 1955].

  The NOEL in this study for microscopic pathological changes was 28 mg/kg/day at day 84 [Monsanto, 1983].

- In a chronic study to investigate the effects of dietary ethoxyquin and vitamin E supplementation with respect to food intake and body weight gain over a period of 500 days, three groups of 5 male weanling Wistar rats were fed a powdered diet containing 0.5% ethoxyquin, and 0.5% or

0.05% DL-tocopheryl acetate in antioxidant-free arachis oil. Controls received powdered diet with 2.5% arachis oil only. Rats eating 0.5% ethoxyquin ate less than controls and experienced a decreased growth rate. After 280 days, 4 animals died from severe kidney failure [Rudra et al., 1974].

Numerous studies have been conducted on the carcinogenic promoting and inhibitory effects of ethoxyquin. However, few studies have been conducted on the carcinogenic potential of ethoxyquin alone. For the purposes of this summary, studies on the inhibitory effects of ethoxyquin were omitted.

- Infant male and female Swiss albino (ICR/Ha) mice were divided into 3 groups as follows: group 1 (57), group 2 (53), and group 3 (28) mice. Group 1 mice were injected subcutaneously with 1 mg ethoxyquin in tricaprylin on days 1 and 7, and 2 mg ethoxyquin on days 14 and 21. Group 2 mice were injected with 5 mg ethoxyquin on days 1 and 7, followed by 10 mg of ethoxyquin on days 14 and 21. Group 3 mice received 10 mg of ethoxyquin subcutaneously on day 1. Control mice received solvent injections or were not injected.

  The percent of mortality prior to weaning in groups 1, 2, and 3 was 2%, 74% and 100% respectively. Mortality prior to weaning was 14% and 19% in the injected and uninjected controls, respectively. Relative to controls, there was no evidence of weight loss in any group. Surviving mice were sacrificed at 49 and 53 weeks.

  In the group administered a total of 30 milligrams of ethoxyquin, one male and one female had an increase and a dose-related occurrence of solitary pulmonary adenomas and malignant lymphoma. The occurrence of lymphomas in test groups was enhanced in contrast to controls as follows: 4/31 in the females of the low ethoxyquin dose group, and 2/9 and 2/5 in the males and females in the high dose ethoxyquin group.

  The authors concluded that the observed increase and dose-related occurrence of solitary adenomas and lymphomas in both sexes is of interest and is consistent with similar findings in adult rats after feeding with a related compound, Flectol-H (a polymer of 1,2-dihydro-2,2,4-trimethylquinoline). However, the authors also noted that an increase in solitary tumors is an unreliable carcinogenic index, especially when unaccompanied by multiple adenomas in test animals [Epstein et al., 1970].

- Groups of 15 male and 15 female A/He mice were used in a study to test the carcinogenicity of 41 food additives and 20 chemotherapeutic agents. The maximum single dose that all mice tolerated after receiving 6 intraperitoneal injections over a 2-week period was determined for all test chemicals. Two groups of mice were injected intraperitoneally 3 times per week for a total of 24 doses with 12 and 2.4 g/kg ethoxyquin in tricaprylin. Positive controls received 2 dose levels of urethan (5 or 20 mg/mouse). Untreated mice and mice treated with tricaprylin served as

controls. Twenty-four hours after the first injection, animals were sacrificed. One male and 4 females in the first group (12 g/kg) and 3 males and 3 females in the second group (2.4 g/kg) developed lung tumors. These incidences, expressed as number of lung tumors per mouse, were as follows: 0.07 ± 0.02 (M, 12 grams); 0.27 ± 0.07 (F, 12 grams); 0.21 ± 0.06 (M, 2.4 grams); and 0.27 ± 0.07 (F, 2.4 grams). In contrast, mice receiving the positive control urethan (5 mg/mouse) demonstrated tumor incidences of 10.5 ± 2.30 (M, 10 grams), 9.1 ± 2.28 (F, 10 grams). At 20 milligram urethan dose levels, males had 21.8 ± 4.48 tumors/mouse and females had 19.6 ± 4.20 tumors/mouse. All mice treated with urethan developed lung tumors. Among the untreated controls (n=50), 22% of the male mice and 17% of the female mice developed tumors (incidence rate of 0.22 ± 0.03 and 0.17 ± 0.02, respectively). Among tricaprylin controls (n=80), 28% of the males and 20% of the females developed pulmonary adenomas. Incidence rates were 0.24 ± 0.03 for males and 0.20 ± 0.02 for females. Based on these results, ethoxyquin was determined to be non-carcinogenic in the strain A mouse assay [Stoner, et al., 1973].

- Four groups of male Fischer 344 rats were used in a study to determine whether ethoxyquin would prevent the formation of pre-neoplastic lesions induced by Aflatoxin B_1 (AFB_1). Eight rats were used per group with the exception of group 3 which had 10 rats. Group 1 was fed a control diet containing 50% peanut oil combined with 50% powdered lab chow containing 2% arachis oil. Group 2 received single intraperitoneal injections of 0.25 mg/kg Aflatoxin B_1 (AFB_1), followed by a toxic diet (peanut meal containing naturally contaminated AFB_1 blended to a 1 ppm concentration). Group 3 received the same diet as group 2, but was pre-exposed to a control diet containing 0.5% ethoxyquin maintained throughout the experiment. Group 4 received the control diet containing ethoxyquin. At 23 weeks, all animals were sacrificed.

  The mean body weight of all groups was found to be lower than respective controls, however, this difference was only significant for group 3 ($p<0.001$). Liver weights were found to be significantly increased in rats from group 3 ($p<0.05$) and group 4 ($p<0.001$). Kidney weights also increased in groups 3 and 4, but not significantly.

  In rats fed ethoxyquin and AFB_1 in the diet, a complete prevention of the formation of AFB_1-induced pre-neoplastic lesions was observed as evidenced by the presence of morphological alterations, and markers such as gamma glutamyl transpeptidase, glutathione S-transferase, and P or J1 (unknown membrane bound antigens).

  Histological examination revealed that kidneys from ethoxyquin-treated animals, with or without AFB_1, demonstrated many characteristics of chronic glomerulonephrosis characteristic of old animals, as well as unusual lesions. Some tubules were hyperplastic and had large amounts of brown pigment and mitotoic figures. Others had putative pre-neoplastic kidneys. Kidneys from the ethoxyquin/AFB_1 animals had

similar but more extensive damage. Large globules composed of lipofuscin were seen. Guanidinobenzoatase (GB-ase), a proteolytic enzyme that is not normally seen in rat kidneys but is associated with alterations induced in cells by carcinogens, was found in hyperplastic tubules. Gamma glutamyl transpeptidase activity was decreased in ethoxyquin-treated animals, and an even more pronounced decrease among ethoxyquin/AFB_1 treated animals was seen.

Formation of pre-neoplastic lesions by AFB_1 was prevented by treatment with ethoxyquin. However, ethoxyquin treatment alone caused marked periportal injury. Ethoxyquin appeared to accelerate the kidney aging process in rats. Ethoxyquin treatment alone resulted in formation of hyperplastic tubules and small nodules of epithelial proliferation, which may be precursors of adenomas [Manson et al.,1987].

The studies which follow concern the induction of tumor formation by ethoxyquin. A summary of this data is presented in Table 2.

TABLE 2.
Ethoxyquin Induction Tumor Formation in F344 Rats

SITE	CARCINOGENIC AGENT	PERCENT ETHOXYQUIN IN DIET (DAYS)	INCREASE TUMOR FORMATION (YES/NO)	REFERENCE
Forestomach	BNA	0.25% (7)	YES	Hirose, et al., 1986
Urinary Bladder	BNN	0.5-0.125% (154)	NO	Fukishima, et al., 1987
Urinary Bladder	BNN	0.8% (224)	YES	Fukishima, et al., 1987
Urinary Bladder	BNN	0.8% (22)	YES	Miyata, et al., 1985
Urinary Bladder	BNN	0.8% (203)	YES	Ito, et al., 1986
Glandular Stomach	MNNG	1.0% (56)	YES	Takahashi, et al., 1986
Esophageal	DBN	0.8% (252)	YES	Fukishima, et al., 1987
Distal Colon	DMH	0.8% (252)	NO	Ito, et al., 1986
Kidney	EHEN	0.8% (203)	YES	Ito, et al., 1986

- In a study examining the induction of forestomach lesions by ethoxyquin, 5 groups of male F344 rats were pre-treated with 0.25% ethoxyquin in a basal diet for 1 week. During the second week, butylated hydroxy-anisole (BHA) was also added to the test diet, and food intake was measured. BHA-induced epithelial hyperplasia in the forestomach was found to be enhanced by ethoxyquin. The group receiving BHA/ethoxyquin had pronounced epithelial damage, including hyperkeratosis and ulcer formation in the forestomach. Severe hyperplasia was noted in one rat in the BHA/ethoxyquin group ($p<0.01$). Ulcers were also observed in rats treated with BHA and ethoxyquin [Hirose, et al., 1986].

- Male F344 rats were divided into 13 groups. Groups 1-10 were given drinking water with or without 0.05% N-butyl-N-(4-hydroxylbutyl)

nitrosamine (BBN) for 2 weeks. For 22 weeks the rats were given Oriental M chow containing 0.5%, 0.25% or 0.125 % ethoxyquin (groups 7, 9, 13) or no chemical (control group 10). On day 22, the lower section of the left ureter was ligated from all rats. At week 24, all animals were sacrificed. A dose-dependent decrease in pre-neoplastic lesions in the bladder of BBN treated rats was seen in ethoxyquin-exposed animals. The authors report that "no promoting activity or induction of pre-neoplastic lesions in the urinary bladder occurred even at the highest ethoxyquin dose (0.5%)." However, the authors also note that other studies using 0.8% ethoxyquin demonstrated promoting activity [Fukushima et al., 1987].

- Male F344 rats were divided into 7 groups of 25 rats each. Groups 1-4 were given drinking water with 0.05% BBN, and groups 5-7 were given water without BBN for 4 weeks. Rats were then fed a basal diet containing 0.8% ethoxyquin (groups 3 and 7) for 32 weeks. Group 4 (control group) received no antioxidant. At 36 weeks, rats were sacrificed and liver and kidneys weighed and analyzed. No clinical abnormalities were observed. BBN/ethoxyquin treated rats had low body weights at week 4 compared to controls. Upon histological examination, simple hyperplasia was found in rats in group 3. Lesions of the urinary bladder were significantly higher in group 3 ($p<0.001$). Incidence and number of cancers were slightly increased in group 3 versus controls, although not significantly. In the absence of BBN, ethoxyquin induced papillary or nodular hyperplasia at a low incidence. The authors concluded that ethoxyquin alone induced pre-neoplastic lesions of the urinary bladder and thus may be weakly carcinogenic to the urinary bladder in a long-term carcinogenicity test [Fukishima et al., 1984].

- Five hundred and eighty male F344 rats were divided into 35 groups and used for a short-term screen for promoters of bladder carcinogenesis in BBN unilaterally ureter-ligated rats. Animals were given drinking water with (groups 1-18) or without (groups 19-35) 0.05% BBN for 2 weeks. Rats were then fed a basal diet containing 0.8% ethoxyquin (groups 9 and 26) for 22 weeks. Group 1 served as the control group and received no test compound. On day 22, the left ureter of all animals was ligated. At week 24, animals were sacrificed. Histological examination revealed incidences and quantitative values of hyperplasia significantly higher in group 9 ($p<0.05$) indicating significant promoting activities in the induction of pre-neoplastic lesions of the urinary bladder. No incidence of hyperplasia were seen in group 26. Ethoxyquin was classified as a weak promotor of bladder carcinogenesis [Miyata et al., 1985].

- Wistar rats were divided into 12 groups, 20 animals per group. Groups 1-6 were given N-methyl-N-Nitro-N-nitrosoquanidine (MNNG) in drinking water at 100 mg/l and a diet containing 10% sodium chloride for the first eight weeks. At week 8, the carcinogenic solution was replaced with tap water and rats were maintained on a basal diet containing 1% ethoxyquin (Group 5). Group 1 served as control and received no ethoxyquin. Groups 7-12 were not fed carcinogenic MNNG or sodium chloride in their diet for the first 8 weeks and were given antioxidant only and served as

controls to groups 1-6. (Group 11 received ethoxyquin.) After 40 weeks, animals were sacrificed and autopsied. Compared with the group given MNNG alone, the incidence of gastric tumors was significantly increased in animals given ethoxyquin ($p<0.05$), after MNNG. Upon examination of the organs, the kidneys of the ethoxyquin-treated rats developed lesions modified by MNNG with sodium chloride pretreatment. Nephrosis of kidneys in rats given ethoxyquin alone microscopically revealed marked deposition of brown pigments in the proximal tubules. Ethoxyquin treatment following initiation with MNNG resulted in pigments that were small and granular in appearance. The pelvic area had small translucent crystals. Histological lesions were advanced, and calcification was observed. Results of this experiment indicate that ethoxyquin is capable of enhancing tumor induction in the glandular stomach [Takahashi et al., 1986].

- Male F344 rats were divided into 11 groups of 20-21 rats per group. In the first 4 weeks, groups 1-6 were given drinking water with 0.05% N, N-dibutylnitrosamine (DBN) in dark bottles, and a basal diet containing 0.8% ethoxyquin (group 3) for 36 weeks. Group 6 (control) received no test compound. Groups 7-11 were given drinking water without DBN for 4 weeks, and a basal diet containing corresponding test compound. After 36 weeks, rats were sacrificed. Mean body weights of test groups 1-5 were lower than controls after week 2, with a slight reduction in food intake seen in groups 2-5 compared to group 6. Incidence of esophageal papillomas was significantly higher ($p<0.05$) in groups 2 and 3 versus group 6. The authors concluded that ethoxyquin significantly enhanced the induction of esophageal papillomas by DBN [Fukishima et al., 1987].

- The effects of antioxidants on colon tumor development were studied using male F344 rats. Rats were given subcutaneous injections of 1,2-dimethylhydrazine (DMH) at a dose of 20 mg/kg once per week, for 4 weeks. One week after the last injection, rats were placed on a diet containing 0.8% ethoxyquin for 36 weeks. At week 40, rats were sacrificed. Ethoxyquin did not affect the incidence of distal colon tumors. However, the number of tumors per rat were significantly (p value not specified) increased by ethoxyquin [Ito et al., 1986].

- The effects of antioxidants on urinary bladder carcinogenesis were investigated in F344 rats. Rats were given drinking water containing 0.05% N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN) for 4 weeks followed by administration of 0.8% ethoxyquin in the diet for 32 weeks. Administration of ethoxyquin significantly increased ($p<0.001$) the incidence of papillary or nodular hyperplasia [Ito et al., 1986].

- In a similar study, male F344 rats were given 0.1% N-ethyl-N-hydroxyethylnitrosamine (EHEN) in drinking water for 2 weeks. After cessation, powdered diet was given with 0.8% ethoxyquin for 29 weeks. Rats which did not receive EHEN were given ethoxyquin from week 4 until the end of the experiment. At week 32, animals were sacrificed. The incidence of renal-cell adenomas was significantly increased in ethoxyquin groups ($p<0.01$) compared to controls. The authors concluded

that ethoxyquin promoted induction of EHEN-initiated kidney adenomas [Ito, 1986].

- In a study concerning the tumor promoting effects of ethoxyquin on the urinary bladder, 195 male F344 rats were divided into 13 groups of 15 rats per group. Each group was fed a basal diet containing a tumor promoter; one group was fed basal diet containing 0.8% ethoxyquin. A control group received basal diet with no test chemical. At week 4, body weight was less in the group receiving ethoxyquin compared to controls. In ethoxyquin treated rats, the lumina surface of the urinary bladder had irregular-shaped foci with a slight elevation of cells, giving the mucosa a cobblestone appearance. Scanning electron microscopy revealed pleomorphic microvilli, short uniform microvilli and ropy or leafy microridges [Fukushima et al., 1986].

E. Reproductive Effects and Teratogenicity

1. Human Data

No data were found on the reproductive or teratogenic effects of ethoxyquin on humans.

2. Animal Data

- Groups of 12, 60-day-old female Sprague-Dawley rats were administered ethoxyquin at concentrations of 0%, 0.025%, 0.05%, and 0.1% in a tocopherol-deficient diet. At 100 days of age, the rats were mated. Animals receiving 0.1% ethoxyquin were discarded from the study because this concentration seemed "unnecessarily high." Two more successive matings were conducted after weaning, and the offspring of the first mating were mated at 100 days of age for a second generation study. The investigators concluded that the animals receiving experimental diets produced young and raised them more successfully than controls. This was attributed to the difference in ability of ethoxyquin-fed animals to withstand the stress of successive matings with low vitamin E, compared to controls [Western Utilization Research Branch, 1956].

- In a teratogenicity study, pregnant female Wistar rats (20 per group) received the following test doses of ethoxyquin suspended in corn oil once per day by esophageal intubation: 0, 125, 250, or 500 mg/kg from day 6 to 15 of gestation. On the 22nd day of gestation, dams were killed and uterine contents were removed and analyzed. No signs of toxicity or any adverse effects were seen in the dams. Resorption of fetuses at 125 mg/kg ethoxyquin was increased, although not significantly. The incidence of anomalous fetuses in the 250 mg/kg group was borderline significant ($p=0.05$). The authors noted that this was most likely not significant in relation to the absence of significant findings in the 500 mg/kg dose group [Khera et al., 1979].

- In the first half of a reproduction study, young female Sprague-Dawley

rats, 1-10 days pregnant, were placed on a basal diet containing ethoxyquin at 125 ppm (group 2), 375 ppm (group 3), and 1125 ppm (group 4). The control group (group 1), received no test compound. The basal diet contained a synthetic vitamin mix. No adverse effects were observed.

In the second part of this study, 3 young adult virgin female rats and 1 male Sprague-Dawley rat were placed in a cage and fed lab chow. Upon evidence of fertile mating, females were isolated and given a test diet containing 0, 125, 375 or 1125 ppm ethoxyquin. A reduced number of offspring was observed in the 1125 ppm ethoxyquin group (50%) compared to controls. The number of live offspring at day 3 was slightly less in the 125 ppm group (70.3%), the 375 ppm group (74.3%), and the 1125 ppm ethoxyquin group (66.7%) compared to control groups (80.5%). No maternal toxicity or other adverse effects were observed in this study [Wisconsin Alumni Research Foundation, Biochemical Laboratory, 1956].

- New Zealand white female rabbits were divided into 3 groups, 16-20 rabbits per group. Group 1 was fed rabbit feed containing 0.0025% ethoxyquin, group 2 received commercial feed without ethoxyquin, and group 3 was given the same feed with 0.0050% ethoxyquin, for at least 10 days before breeding to at least 2 weeks after parturition. Reproducing males were given the same rations 10 days before breeding.

  Some rabbits from the 3 groups described above were used in the following experiment: Group 1 (14 rabbits) were fed rabbit food containing 0.0025% ethoxyquin; group 2 (16 rabbits) were fed feed without ethoxyquin; and group 3 (11 rabbits) were fed feed containing 0.0100% ethoxyquin. Unlike the rabbits from the first part of this study, rabbits were housed in a heated building just before parturition and for 2 weeks after parturition.

  The investigators reported that the average litter size ranged from 6.3 to 7.7 for all six groups. They also found that abortions did not occur, and that physical deformities were not observed in any rabbit delivered alive or dead. The percentage of mortality at birth and the percentage of total mortality were higher for the non-ethoxyquin-treated group versus the treated group in experiment 2 only. Mortality after the first and second weeks of birth was higher among rabbits fed ethoxyquin versus non-treated rabbits in experiment 1, although the observed increase was not significant [Isenstein, 1970].

- Three hundred hatched unsexed SCWL chicks were fed the following test diet: group 1 (230 chicks) received 5% alfalfa, group 2 (221 chicks) received 5% alfalfa combined with 0.05 % ethoxyquin, group 3 (223 chicks) received 5 % alfalfa combined with 0.15% ethoxyquin, and group 4 (227 chicks) received 5% alfalfa combined with 1.5% ethoxyquin for 10 weeks, for final ethoxyquin concentrations of 0, 0.00075%, 0.0075%, and 0.075%. Viability and growth were the same for all groups during the study. From the above test groups, 20 cockerels and 100 pullets were

selected and fed the same diet for an additional 10 weeks. A breeding flock was then selected, including 7 cockerels and 50 pullets. The breeding flock was continued on the diet and allowed to reproduce. The authors concluded that ethoxyquin did not effect body weight, livability, or egg production. However, spring hatch fertility and hatchability were lower in the treated groups. The authors concluded that this effect could be associated with neck mold experienced that season. Fall hatch treated groups experienced a small but insignificant decrease in fertility and hatchability compared to spring hatch treated groups [Western Utilization Research Branch, 1954].

To determine the effects of ethoxyquin treatment on male fertility, half the untreated males in the control group were exchanged with half the treated males in group 4 two weeks before collecting fall hatched eggs. The remaining chicks served as controls. Eggs were collected and hatched at weekly intervals. Fertility hatch was high in the treated males and control females and lower in treated females and control males, indicating a high rate of embryonic death.

The duration of fertilizing capacity of spermatozoa remaining in the hens oviduct was also determined. Fertility was decreased in all groups within 3 weeks of removal of cockerels. Group 1 fertility had decreased from 70% to 8%, group 2 fertility had decreased from 88% to 27%, group 3 fertility had decreased from 78% to 7%, and group 4 fertility had decreased from 79% to 12 %. An overall decrease from 78% to 11% in fertility occurred. Eggs collected during the fourth week were infertile [Western Utilization Research Branch, 1954].

F. Genetic Toxicology

1. Human Data

No data were found on the genetic effects of ethoxyquin on humans.

2. Eukaryotic Data

- In the Ames microsome mutagenicity test on *Salmonella typhimurium* strains TA98 and TA100, ethoxyquin (concentrations unspecified) was mutagenic in the presence of metabolic activation [Hedenstedt, 1981].

- In the Ames test, ethoxyquin (concentrations unspecified) induced frameshift mutations in *Salmonella typhimurium* strains TA1538 and TA98 when tested in the presence of a metabolizing system containing NADP. No other data were available [Hedenstedt, 1982].

- In the Ames test, with S9 metabolizing mixture, plus cofactors, ethoxyquin was mutagenic to *Salmonella typhimurium* strains TA1538 and TA98 at 200 µg per plate. However, ethoxyquin was non-mutagenic to strain TA1535. Ethoxyquin was classified as a weak mutagen, inducing revertants at or above 50 µg per plate [Rannug et al, 1984].

- In the Ames test using *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100 (with and without addition of microsomal mixed function oxidase system from rat liver) ethoxyquin was tested in concentrations of 10-1000 μg per plate. Ethoxyquin demonstrated no mutagenic activity. However, at high concentrations ethoxyquin demonstrated toxic effects on the bacteria [Joner, 1977].

- Ethoxyquin was tested in the rec-assay for DNA modifying effects in *Bacillus subtillis* H17 (rec+) and M45 (rec -). In addition, ethoxyquin was tested for mutagenicity in *Escherichia coli* WP2 hcr and *Salmonella typhimurium* strains TA1535, TA100, TA1537, TA1538 and TA98 in the presence and absence of metabolic activation. Ethoxyquin was negative in the rec-assay. In addition, this compound was non-mutagenic to *E. coli* and all strains of *S. typhimurium* tested at concentrations up to 5 milligrams per plate [Ohta et al., 1980].

- As described in a German abstract, the SOS chromotest was used to evaluate the genotoxicity of antioxidants including ethoxyquin. Ethoxyquin was non-mutagenic in this test system. No other data was provided in this report [Von der Hude, et al., 1987].

- In the Ames mammalian microsome test, with *S. typhimurium* strains TA100 and TA98, 3,2-dimethyl-4-aminobiphenyl (DMAB) dissolved in dimethyl sulfoxide (DMSO) was added in addition to liver microsomal S-9 mix. Ethoxyquin alone was not mutagenic to TA98 or TA100 with or without S-9 mix. Ethoxyquin enhanced the DMAB-induced mutagenic activity at 50-250 μg per plate in TA100 and at 50 μg per plate in strain TA98. No effect was seen at the 125 and 250 μg levels in TA98 [Reddy et al., 1983].

G. Other Toxicological Effects

1. Immunotoxicity

No human data were found on the immunotoxic effects of ethoxyquin.

The following animal study has been described:

- C57B1/6 female mice were placed on selenium deficient (0.005 ppm) and selenium sufficient (1.0 ppm) diets. Two additional selenium sufficient mice received ethoxyquin in the diet at 0.02 and 0.25% for 60 days. After 56 days, mice were injected intravenously with a 25% suspension of sheep erythrocytes, and the immune response to this antigenic challenge was quantitated at 60 days. The 0.02% ethoxyquin group experienced no significant effect on immunosuppression. The 0.25% ethoxyquin dose reduced immune response to the level of animals receiving selenium deficient diets ($p<0.05$). Serum selenium, liver DNA, liver RNA, and liver protein levels were the same in all dose groups. The authors concluded the ethoxyquin in the diet did not reduce general tissue function in a non-specific manner, but rather, this compound is immunosuppressive by other unknown mechanisms [Travis, et al., 1989].

2. Neurotoxicity

No data were found on the neurotoxic effects of ethoxyquin in animals or humans.

3. Biochemical Toxicology

a. Human Data

No data were found on the biochemical toxicology of ethoxyquin in humans.

b. Animal Data

- Male Wistar rats were fed a conventional diet containing 1% ethoxyquin for 14 days. Animals were sacrificed 36 hours after ethoxyquin was removed from the diet. Concentration- and NADPH-dependent hydrogen peroxide formation was enhanced in rat liver microsomes, although not significantly. Hydrogen peroxide doubling occurred in lung microsomes pretreated with phenobarbital at 500 μM ethoxyquin [Rossing et al., 1985].

  The *in vivo* effects of ethoxyquin on liver homogenates prepared from male Wister rats as described above have been studied. A decrease in oxycytochrome P-450 concentration from 0.138 ± 0.020 to 0.073 ± 0.010 nmol/mg protein ($p<0.02$) was observed, but no increase in hydrogen peroxide formation in liver microsomes occurred. The authors report that the data suggest that ethoxyquin enhances the oxidase function of cytochrome P-450 [Rossing et al., 1985].

- Male Sprague-Dawley rats were administered a powdered diet containing 0.5% ethoxyquin for 14 days. The supplemented diet was replaced with a control diet 24 hours prior to sacrificing. One hundred milligrams of phenobarbital were administered interperitoneally followed by 0.1% (w/v) phenobarbital in drinking water for 4 days. In addition, a dose of 40 mg/kg 3-methylcholanthracene in olive oil was administered intraperitoneally. Ethoxyquin was found to stimulate 3-methylcholanthracene and phenobarbital-inducible glucuronidation. However, phenobarbital inducible bilirubin glucuronidation was not affected by ethoxyquin [Bock et al., 1980].

  The inhibition of rat hepatic microsomal enzymes by ethoxyquin was investigated in the following studies:

- Four rats were fed 2.5% ethoxyquin-free arachis oil in Spillers animal diet. At 48 days, animals were sacrificed. Ethoxyquin was added to microsomal preparations (220 mM solution in ethanol-water). Ethanol only was added to the control diet. Competitive inhibition of biphenyl 4-hydroxylase and ethylmorphine N-demethylase occurred in the ethoxyquin test. No inhibitory activity of glucose-6-phosphatase was evident [Parke et al., 1974].

- In a single dose effect study, 56 weanling rats were dosed intragastrically with ethoxyquin at 500 mg/kg as a 25% solution in arachis oil. Control groups received arachis oil only. Immediately after dosing, and 2, 4, 8, 12, 24, and 48 hours after dosing, 8 rats from each group were sacrificed and the livers were prepared and assayed for biphenyl 4-hydroxylase and ethylmorphine N-demethylase activity. Microsomal proteins and cytochrome P450 were also measured. Ethoxyquin was found to significantly affect enzyme activity ($p<0.001$) 24 hours after administration [Parke, et al., 1974a].

- In an inhibition study *in vivo*, rats were given 100 mg/kg hexobarbital by intraperitoneal injection. The rate of metabolism was studied by observing sleeping time intervals after a single intragastric dose of ethoxyquin (500 mg/kg) in arachis oil. Ethoxyquin inhibited hexobarbitone metabolism 50% compared to control rats, 3 hours after withdrawal from the diet. The spectral dissociation constant (ks) of ethoxyquin was determined to be 1.06×10^{-5} M. The authors concluded that the above the results indicate that ethoxyquin binds strongly to P-450 and is a potent inhibitor of drug metabolizing activities of the enzyme [Parke, et al., 1974a].

- The reversibility of hepatic changes caused by ethoxyquin was studied in weanling male Wistar rats. Rats were fed a diet of Spillers small animal chow containing 0.5% ethoxyquin in arachis oil for 14 days. The control group was fed the same diet with 2.5% ethoxyquin-free arachis oil. In the test group liver weights were 90% greater ($p<0.001$) than controls (3 day sacrifice). The 35% decrease in DNA concentration compared to a 25% increase in total hepatic DNA observed indicated that liver enlargement may be due to cell hypertrophy and hyperplasia. Significant increases ($p<0.01$) were observed in concentration and total amount of microsomal protein (cytochrome P-450, cytochrome b_5, and biphenyl-4-hydroxylase activity) in the liver after the administration of ethoxyquin. Glucose-6-phosphatase levels were not affected. At the end of a 30 day recovery, liver size and DNA content appeared normal, suggesting reversible liver damage. Recovery from hyperplasia and cytochrome P-450 elevation was less rapid. The authors state that the complete reversibility of the hepatic changes produced in rats by dietary ethoxyquin suggest that the observed hepatomegaly and enzyme induction should be considered an adaptive response [Parke et al., 1974b].

- A study was conducted to measure the induction of liver mRNA during non-carcinogenic treatment with ethoxyquin and during aflatoxin B_1 (AFB_1) induced hepatocarcinogenasis in rats. Male F344 rats were fed 0.5% ethoxyquin in their diet, or a diet containing naturally occurring peanut meal contaminated with AFB_1 at 1 or 4 ppm. In the untreated liver, glutamyltranspeptidase (GGT) was present in the bile duct only. Ethoxyquin treatment induced GGT in the periportal to midzonal regions of the liver lobules during AFB_1-induced carcinogenesis. GGT was present in the cortex of untreated kidneys. GGT mRNA was detected in all treated liver samples and the hepatoma-derived JB_1 cell line, but not

in control livers. Kidney mRNA levels were generally higher than liver levels. In general, mRNA levels corresponded well with enzyme activities [Power et al., 1987].

- Six radical reaction inhibitors were investigated for their effects on rat liver mitochondria. Ethoxyquin dissolved in dimethyl sulfoxide had a marked effect on mitochondrial function. Ethoxyquin at 500 μM altered succinate and glutamate-malate mitochondrial respiration, and increased resting state respiration (2-fold compared to controls ($p<0.001$)). Ethoxyquin altered both the glutamate respiration which proceeds through complex I (NADH-coenzyme Q reductase system) and succinate respiration which proceeds through complex II (succinate-coenzyme Q reductase system), suggesting that inhibition may occur at a common site, coenzyme Q. This elevated rate was unaffected by either adenosine diphosphate (ADP) or uncoupler p-trifluoromethoxyphenol hydrazone (FCCP), indicating an uncoupled-like state [Horrum et al, 1987].

- Male Sprague Dawley rats were fed powdered Altromin diet containing 1% ethoxyquin for 14 days. A slow marked induction of epoxide hydratase activity was observed [Kahl R., 1980].

- The effects of ethoxyquin on renal production of prostaglandins and cAMP was investigated in Sprague-Dawley rat inner medula slices. Slices were incubated with test agents including ethoxyquin, and then assayed for prostaglandin E_2 (PGE_2), progtaglandin F_2 (PGF_2), and cyclic AMP levels. Ethoxyquin (0.01 and 0.001 mM) caused a 50% significant ($p<0.05$) reduction in PGE_2. Also, ethoxyquin decreased ($p<0.05$) PGE_2 production in the presence of arachidonic acid. Ethoxyquin also inhibited PGF_2 synthesis significantly ($p<0.05$) at concentrations of 0.01, and 0.1 mM. Ethoxyquin did not alter cAMP levels [Zenser et al., 1978].

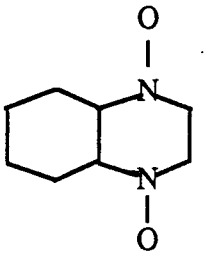
VI. STRUCTURE ACTIVITY RELATIONSHIPS

Hodge, et al. state, "Although there are no published data on the carcinogenicity of ethoxyquin, a polymer of the closely related compound 1,2-dihydro-2,2,4-trimethylquinoline, Flectol H_4 , which is used as a rubber antioxidant, produced cholangiofibrotic nodules, pulmonary adenomas, and lymphomas after chronic feeding in rats "[Epstein S.S., 1970]. In addition, ethoxyquin is structurally similar to Quindoxin, a known carcinogen [Zachariae, 1978]. The structures of Quindoxin and Flectol H_4 are shown on the following page.

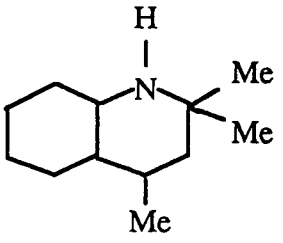
No other data were found on structure activity relationships for ethoxyquin.

The structures of Quindoxin and Flectol H_4 are shown below.

Quindoxin



Flectol H_4



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APPENDIX I. ON-LINE DATABASES SEARCHED

	DATE OF SEARCH	TIME PERIOD
BRS:		
HZDB	April, 1990	
DIALOG:		
Agricola	April, 1990	1970-1990
Agris International	April, 1990	1974-1990
Aquatic Science Abstracts	April, 1990	1978-1990
Biosis Previews	April, 1990	1969-1990
Biotechnology Abstracts	April, 1990	1982-1990
CAB Abstracts	April, 1990	1972-1990
Cancerlit	April, 1990	1963-1990
Chemical Engineering Abstracts	April, 1990	1971-1990
Chem Bus Newsbase	April, 1990	1984-1990
Chemical Exposure	April, 1990	1974-1987
Compendex Plus	April, 1990	1970-1990
CRIS USDA	April, 1990	
Embase	April, 1990	1974-1990
Enviroline	April, 1990	1970-1990
Environmental Bibliography	April, 1990	1974-1990
Federal Register	April, 1990	1977-1990
Foods Adlibra	April, 1990	1974-1990
FSTA	April, 1990	1969-1990
Life Sciences Collection	April, 1990	1978-1990
Medline	April, 1990	1966-1990
NTIS	April, 1990	1964-1990
Occupational Safety and Health	April, 1990	1973-1990
Pascal	April, 1990	1984-1990
PTS Newsletter	April, 1990	1987-1990
PTS Prompt	April, 1990	1972-1990
Pollution Abstracts	April, 1990	1970-1990
Scisearch	April, 1990	1974-1990
Trade and Industry ASAP	April, 1990	1983-1990
Trade and Industry Index	April, 1990	1981-1990
World Translations Index	April, 1990	1984-1990
MEAD:		
Nexis/Lexis-BNA ENV	April, 1990	
NLM:		
Chemid	April, 1990	
Chemline	April, 1990	
HSDB	April, 1990	
RTECS	April, 1990	
Toxline 65	April, 1990	1965-1980
Toxline	April, 1990	1981-1990
Toxlit	April, 1990	1981-1990
Toxlit 65	April, 1990	1965-1980
STN:		
Beilstein	April, 1990	
CA	April, 1990	1967-1990
Chemlist	April, 1990	
Registry	April, 1990	

APPENDIX II. SAFETY INFORMATION

- **HANDLING AND STORAGE**

  Ethoxyquin is stable under normal laboratory conditions.

- **EMERGENCY FIRST AID PROCEDURES**

  Eye: First check the victim for contact lenses and remove if present. Flush victim's eyes with water or normal saline solution for 20 to 30 minutes while simultaneously calling a hospital or poison control center. Do not put any ointments, oils, or medication in the victim's eyes without specific instructions from a physician. Immediately transport the victim to a hospital even if no symptoms (such as redness or irritation) develop.

  Skin: IMMEDIATELY flood affected skin with water while removing and isolating all contaminated clothing. Gently wash affected skin areas thoroughly with soap and water. If symptoms such as inflammation or irritation develop, IMMEDIATELY call a physician or go to a hospital for treatment.

Inhalation: IMMEDIATELY leave the contaminated area and take deep breaths of fresh air. If symptoms (such as wheezing, coughing, shortness of breath, or burning in the mouth, throat, or chest) develop, call a physician and be prepared to transport the victim to a hospital.

Provide proper respiratory protection to rescuers entering an unknown atmosphere. Whenever possible, Self-Contained Breathing Apparatus (SCBA) should be used.

Ingestion: If the victim is conscious and not convulsing, give 1 or 2 glasses of water to dilute the chemical and IMMEDIATELY call a hospital or poison control center. Be prepared to transport the victim to a hospital if advised by a physician.

If the victim is convulsing or unconscious, do not give anything by mouth, ensure that the victim's airway is open, and lay the victim on his/her side with the head lower than the body. DO NOT INDUCE VOMITING. IMMEDIATELY TRANSPORT THE VICTIM TO A HOSPITAL.

- **PROTECTIVE EQUIPMENT**

  Eye: Safety glasses

  Gloves: Two pairs of dissimilar protective gloves shall be worn when handling the neat chemical, otherwise one pair. When contact with this chemical has been known to occur, change gloves immediately.

  Clothing: Minimally, a disposable laboratory suit (e.g. Tyvek ®) shall be worn, as specified in the most current NTP Statement of Work or NTP Health and Safety Minimum Requirements.

  Respiratory Protection: A NIOSH-approved chemical cartridge respirator with an organic vapor cartridge.

- **EXTINGUISHANT**

  Dry chemical, carbon dioxide or halon extinguisher

- **MONITORING PROCEDURES**

  There is no NIOSH analytical method reported in the NIOSH Manual of Analytical Methods for ethoxyquin.

- **SPILLS AND LEAKAGE**

  Persons not wearing the appropriate protective equipment and clothing shall be restricted from areas of spills until cleanup has been completed. When exposure to unknown concentrations may occur, air-purifying respirators may not be used. Chemical cartridge respirators with organic vapor cartridges may not be used when airborne concentrations exceed 1000 ppm.

  If ethoxyquin is spilled the following steps shall be taken:

  1. If a liquid solution is spilled, use vermiculite, sodium bicarbonate, sand, or paper towels to contain and absorb the spill.

  2. Clean the spill area with dilute alcohol (approximately 60-70%) followed by a strong soap and warm water washing.

  3. Dispose of all absorbed material as hazardous waste.

- **DECONTAMINATION OF LABORATORY EQUIPMENT**

  TDMS Terminal: Whenever feasible, a protective covering (e.g., plastic wrap) shall be placed over the keyboard when in use.

General Equipment: Before removing general laboratory equipment (i.e., lab carts, portable hoods and balances) from animal dosing rooms and/or chemical preparation areas, a decontamination process shall be conducted in addition to routine housekeeping procedures.

- **WASTE MANAGEMENT AND DISPOSAL PROCEDURES**

Waste Management: If an inhalation study is to be conducted, all exhaust air from the inhalation chamber must be cleaned with appropriate air cleaning devices unless the laboratory has informed local and state air pollution regulatory agencies of both the laboratory's operating practices and the potential hazards of the chemicals in use. Compliance with all federal, state, and local air pollution laws and regulations is required. A specific air cleaning system design must consider the specific conditions of the laboratory (eg., air flow rates and volumes, mixing of exhaust streams, size of inhalation chamber) and the dosing regimen selected. Air cleaning systems designs must be described by the laboratory and approved by the NTP Office of Laboratory Health and Safety.

Waste Disposal: Securely package and label, in double bags, all waste material. All potentially contaminated material (i.e., carcasses, bedding, disposable cages, labware) shall be disposed of by incineration in a manner consistent with federal (EPA), state, and local regulations or disposed of in a licensed hazardous waste landfill.