

ETHOXYQUIN

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Explanation

Ethoxyquin was previously evaluated by the Joint Meeting in 1969 (Annex 1, reference 12), when an ADI of 0–0.06 mg/kg bw was established on the basis of the NOAELs in a long-term feeding study in dogs and a study of reproductive toxicity in rats. The compound was reviewed at the present meeting within the CCPR periodic review programme. This monograph summarizes new data and data not previously reviewed on ethoxyquin and relevant data from the previous monograph (Annex 1, reference 13).

Evaluation for Acceptable Daily Intake

1. Biochemical aspects

(a) Absorption, distribution, and excretion

Data from the 1950s were summarized briefly in the 1969 JMPR monograph (Annex 1, reference 13). In pretreated rats, equal amounts of a 1.5-mg dose were excreted in urine and faeces, with a total of 64% excreted in 48 h; about 1% of the administered radiolabel was exhaled as carbon dioxide. The liver, kidneys, fat, and skeletal muscle contained the highest concentrations of residues (1–5 mg/kg). Newborn rats had tissue concentrations of 0.12–0.21 mg/kg, indicating some placental transfer; similar concentrations were found in rat milk, but maternal tissue concentrations were not available for comparison. In dogs, excretion occurred primarily in the urine as unidentified metabolites. In chickens, 99% of the radiolabel from an oral dose of ethoxyquin was excreted as metabolites within 48 h; after 12 weeks of administration of about 130 ppm in the diet, the tissue concentrations were low (0.1 mg/kg) and declined rapidly after withdrawal of the treated diet.

The absorption, distribution, and excretion of ethoxyquin were investigated in groups of three male Fischer 344 rats and B6C3F₁ mice, approximately eight weeks old, which received single doses of ethoxyquin (purity, 90%) containing [3-¹⁴C]ethoxyquin (purity, 96%) by gavage at 2.5 (rats only), 25, or 250 mg/kg bw or single intravenous doses of 25 mg/kg bw. The substance was administered in a 1:1:8 mixture of ethanol:emulphor EL620:water at a volume of 1 ml/kg bw, equivalent to 25–50 µCi/kg. Urine, faeces, and air were sampled at various times between 4 and 72 h. The concentrations in liver, kidney, blood, muscle, skin, and adipose tissue were determined by sequential kills between 0.25 and 72 h. Additional groups of four rats were used to determine blood and plasma concentrations in the jugular vein 0.08–24 h after a dose of 25 mg/kg bw and to determine tissue, urine, and faecal concentrations after six doses of 25 or 250 mg/kg bw. Radiolabel was determined by liquid scintillation counting; faecal samples were first powdered and combusted. The concentrations of parent ethoxyquin in the samples were determined by high-performance liquid chromatography (HPLC).

The disposition of ethoxyquin was similar when administered orally and intravenously. It was rapidly absorbed, with peak blood and tissue concentrations within 1 h. Excretion of the oral doses of 2.5 and 25 mg/kg bw was extensive (> 85% within 24 h) and approximately 1.5-fold greater via urine than faeces. The tissue concentrations at 24 h were < 2% of the administered dose (Table 1). Little difference was seen with dose in rats, although the higher dose was excreted more slowly than the lower doses, attributed by the authors to delayed gastric emptying, and there was evidence of significant adipose deposition. The results after three or four repeated doses of 250 mg/kg bw per day were reported to be similar to those after repeated and single dosing at 25 mg/kg bw, indicating induction of metabolizing enzymes and/or a return to normal gastric emptying (data not presented in the published report). The rate of excretion by mice was slightly more rapid than in rats. As parent ethoxyquin was not detectable in plasma at most times, the authors did not calculate its overall bioavailability. About 60% of the radiolabel in blood was in the plasma, and 8% was associated with precipitating plasma proteins. Repeated administration at 25 mg/kg bw per day and, to a lesser extent, 250 mg/kg bw per day to rats was followed by some evidence of bioaccumulation (data not presented) but not in muscle.

After intravenous administration, the highest initial tissue concentrations were found in liver and kidney, although in mice a transiently high concentration was seen in adipose tissue at about 2 h (Table 2). A significant proportion (> 20%) of the intravenous dose was excreted in the faeces in both species (Table 1), and 40% of the administered dose was found in the bile of bile-cannulated rats, indicating that biliary excretion and enterohepatic circulation play a significant role in the toxicokinetics of ethoxyquin. Parent ethoxyquin was not detected in urine and was present in only trace amounts in faeces, liver, kidney, and adipose tissue. The elimination half-life in plasma for parent ethoxyquin was calculated to be 23 min (Sanders et al., 1996).

(b) Biotransformation

Data summarized in the 1969 monograph (Annex 1, reference 13) indicate that the metabolism of ethoxyquin is extensive in rats, dogs, and chickens, although the metabolites were not identified.

Table 1. Tissue distribution at 24 h and excretion over 0–24 h as percent of ¹⁴C-ethoxyquin administered orally or intravenously (i.v.)

Species	Dose (mg/kg bw)	Blood	Liver	Kidney	Muscle	Skin	Adipose tissue	Urine	Faeces
Rat	2.5 (oral)	0.7	1.4	0.3	0.4	0.3	0.9	57	31
	25 (oral)	1	1.3	0.2	0.7	0.4	1.7	64	26
	250 (oral)	0.9	1.6	0.2	1.8	1.2	12	41	11
	25 (i.v.)	1	1.5	0.2	1	0.7	6.4	57	23
Mouse	2.5 (oral)	0.4	1.2	0.1	0.4	0.7	0.6	60	42
	250 (oral)	0.3	1	0.2	1.2	1.2	2.2	43	16
	25 (i.v.)	0.5	1.1	0.2	0.9	1.2	0.9	58	33

From Sanders et al. (1996)
Means of three to six animals

Table 2. Tissue concentrations of ^{14}C at different times after an intravenous dose of ^{14}C -ethoxyquin at 25 mg/kg bw as microgram equivalent per gram

Species	Time (h)	Blood	Liver	Kidney	Muscle	Skin	Adipose tissue
Rat	0.25	6	66	51	9	15	29
	2	5	27	21	2	10	29
	12	2	12	11	< 1	3	24
	24	3	9	10	< 1	1	15
Mouse	0.25	10	45	40	11	27	40
	2	4	27	17	3	16	67
	12	2	9	8	< 1	3	22
	24	2	5	3	< 1	2	2

From Sanders et al. (1996)
Means for three animals

Samples of urine, faeces, and tissues were obtained from rats and mice given [3- ^{14}C]ethoxyquin at 25 or 250 mg/kg bw orally or 25 mg/kg bw intravenously in the study of Sanders et al. (1996), described above. Urine and bile samples were stored frozen, defrosted, and centrifuged before separation by HPLC; liver, kidney, and faecal samples were extracted three times with 1:1:1 water:methanol:ethyl acetate, and the supernatants were separated by HPLC. Plasma samples were mixed 1:1 with acetonitrile prior to centrifugation and investigation by HPLC. The effects of incubation with glucuronidase containing arylsulfatase activity were also studied. Metabolites were investigated by HPLC, ^1H -nuclear magnetic resonance spectroscopy, and three types of mass spectroscopy, including comparison with results for synthesized reference compounds.

Eight metabolites were detected in urine, although only four were characterized (Table 3, Figure 1); no parent ethoxyquin was reported. The main metabolic pathway in both rats and mice seems to involve *O*-deethylation at C-6 followed by conjugation at C-6 with sulfate (metabolite G) or glucuronide (metabolite F). Subsidiary pathways of hydroxylation and glucuronidation at C-8 (metabolite H) or *O*-deethylation at C-6 and epoxidation between C-3,4 with sulfation at C-6 are also indicated. The major difference between rats and mice was the higher levels of glucuronidation in the latter. No significant difference was reported in the metabolite profiles of rats dosed with 25 mg/kg bw orally or intravenously. Administration of ethoxyquin at 250 mg/kg bw resulted in a greater proportion of radiolabel on the C-6 sulfate (metabolite G) than after dosing with 25 mg/kg bw (Table 3). Six doses of 25 mg/kg bw resulted in a urinary metabolite profile similar to that after a single dose. Six doses of 250 mg/kg bw resulted in greater proportions of the glucuronide metabolites F and H and smaller proportions of metabolites G and E than after a single dose, indicating that sulfation may have been saturated or glucuronidation reactions induced. In the kidney and liver, the major metabolite was G. Faecal samples could not be satisfactorily extracted (< 30% recovery), and no reliable conclusions could be drawn. In the bile, three glutathione conjugates were detected, and < 5% of the radiolabel was present as the parent; this

Table 3. Metabolic profile of ^{14}C -ethoxyquin administered by oral gavage to rats, as percent of total radioactivity in 24-h urine sample

Metabolite ^a	Dose (mg/kg bw)			
	1 x 25	6 x 25	1 x 250	6 x 250
A	6	7	4	9
B	6	5	4	7
C	9	8	5	3
D	7	6	2	< 1
E	17	12	10	6
F	5	6	3	15
G	34	42	59	30
H	3	4	4	14
Parent	< 1	< 1	< 1	< 1

From Burka et al. (1996)

^a See Figure 1 for structures

finding is cited as contrasting with the results of other workers, who had reported that most of the biliary radiolabel was present as ethoxyquin. The authors proposed a reaction scheme for the biliary metabolites (Figure 1) which involves production of reactive electrophilic intermediates (epoxides) (Burka et al., 1996).

(c) *Effects on enzymes and other biochemical parameters*

Administration of ethoxyquin (purity unspecified) to male Sprague-Dawley rats at 5000 ppm in the diet for three days significantly induced both phase-1 and phase-2 xenobiotic metabolizing enzymes. Northern blotting of liver preparations for mRNA of cytochrome P450 (CYP) isozymes showed increasing amounts of CYP2B1 > 2B2 > 3A2 > 1A2; assays for enzyme activity showed a twofold increase in the specific activity of CYP1A2 and a 10-fold increase in that of the CYP2B family. Blotting with probes for glutathione *S*-transferase mRNA showed that ethoxyquin increased Ya1, Ya2, and Yb1, with an approximate doubling of the activity of cytosolic glutathione *S*-transferase. Enzyme assays and mRNA blotting also showed increases in NADPH-quinone oxidoreductase, γ -glutamylcysteine synthetase, and UDP-glucuronosyl transferase activities. Ethoxyquin did not alter cellular glutathione concentrations or induce CYP1A1 (Buetler et al., 1995).

Administration of diets containing ethoxyquin (purity unspecified) at 50, 100, 500, 2000, or 5000 ppm to male Sprague-Dawley rats for 14 days induced a range of effects on xenobiotic metabolizing systems. The liver:body weight ratios were increased at 5000 ppm; and total cytochromes P450 and b_5 concentrations were increased by 30% at 2000 and 5000 ppm. Analysis of the CO-reduced microsomal ultraviolet spectra showed that ethoxyquin-treated animals had a λ_{\max} of 449.5 nm, indicating a phenobarbital-type induction pattern rather than a methylcholanthrene-type (λ_{\max} , 448 nm). Monooxygenase activity in microsomes from rats receiving ethoxyquin in the diet at 5000 ppm was increased by 1.5 to 2-fold and epoxide hydratase activity by threefold when styrene oxide was the substrate, but the activities were slightly lower when benzo[*a*]pyrene was used as the substrate. Assays *in vitro* with microsomes from animals induced with phenobarbital or methylcholanthrene showed that ethoxyquin inhibited arylhydrocarbon hydroxylase activity at concentrations of 5 μ mol/L and higher. Animals receiving both ethoxyquin-treated diet (5000 ppm) and methylcholanthrene (three intraperitoneal injections of 20 mg/kg bw) showed no evidence of additive induction of drug metabolizing systems. The NOAEL for changes in xenobiotic metabolizing enzyme systems was 500 ppm, equivalent to 25 mg/kg bw per day (Kahl & Netter, 1977).

2. Toxicological studies

(a) *Acute toxicity*

Ethoxyquin had little acute toxicity, except when administered parenterally (Table 4). The clinical signs of toxicity after exposure to ethoxyquin were tremors, ataxia, hypoactivity, hypothermia, and red–yellow staining of the fur. Gross and histopathological changes indicated an irritant effect on the gastrointestinal tract.

Table 4. Acute toxicity of ethoxyquin

Species	Route	LD ₅₀ or LC ₅₀ (mg/kg bw or mg/L air)	Purity (%)	Reference
Rat	Oral gavage	1700	97.6	Varsho (1995a)
Rat	Dermal (24 h)	> 2000	97.6	Varsho (1995b)
Rat	Inhalation, whole body	> 2.0	97.6	Ulrich (1996)
Mouse	Intraperitoneal	~ 900		Wilson & DeEds (1959) ^a
Mouse	Intravenous	~ 180		Wilson & DeEds (1959) ^a

^a Cited in 1969 JMPR monograph

Ethoxyquin produced transient, slight erythema when applied to rabbit skin for 4 h under semi-occlusive conditions. There was no oedema, but desquamation was present seven days after exposure (Varsho, 1995c). This result is consistent with the findings of a study summarized in the 1969 monograph (Kelly, 1960; cited in 1969 JMPR monograph; Annex 1, reference 13).

Ethoxyquin produced transient, slight-to-mild conjunctival redness and chemosis in rabbits. All of the effects had fully regressed within four days (Varsho, 1995d).

In a sensitization study in six guinea-pigs of each sex, ethoxyquin was at most a very weak skin sensitizer. Induction was at 100%, with challenge applications at 50% in acetone. Very weak erythematous responses were seen in both treated and control groups after challenge and re-challenge, one test animal producing a weak response to both challenge and re-challenge (Varsho, 1995e).

(b) *Short-term studies of toxicity*

Rats

Two studies of dietary administration of ethoxyquin to rats for 200 days were summarized in the 1969 monograph. Kidney lesions (unspecified) were reported at 500 ppm and higher in males, with increased kidney:body weight ratios in males at 250 ppm and higher. The frequency of cytoplasmic inclusions in hepatocytes was increased at 2000 ppm (Wilson & DeEds, 1959; Cox, 1953; cited in 1969 JMPR monograph; Annex 1, reference 13).

In a study described in more detail below of the effects of ethoxyquin on induction of liver tumours by *N*-nitrosodiethylamine, a control group received ethoxyquin only. Thus, 15 male Fischer 344 rats, six weeks old, received an intraperitoneal injection of 0.9% saline, were placed on a diet containing 8000 ppm ethoxyquin (purity unspecified), equivalent to 500 mg/kg bw per day in young rats, in week 2, and were partially (66%) hepatectomized in week 3. When the animals were killed at week 8, a background level of γ -glutamyl transpeptidase (γ -GT)-positive foci was found in the liver (Ito et al., 1985).

Groups of five male and five female Sprague-Dawley rats received ethoxyquin (purity, 97.6%) by gavage in corn oil for 28 days at doses of 0, 50, 250, 500, or 1000 mg/kg bw per day. Histopathological examination was limited to the liver, lung, kidney, stomach, and gross lesions in animals at 50, 250, and 1000 mg/kg bw per day. All of the animals at 1000 mg/kg bw per day had died by day 3 with multiple organ involvement; the cause of death in two animals was considered to be necrosis and ulceration of the forestomach. The prevalences of salivation, stained fur, and brown urine were increased at 250 mg/kg bw per day and higher. Initial body-weight gain was reduced by 50% in males receiving 500 mg/kg bw per day. Erythrocyte count, haematocrit, and haemoglobin concentration were decreased by about 10% in females at 250 mg/kg bw per day and in animals of each sex at 500 mg/kg bw per day. Alterations in serum clinical chemical parameters were seen in both males and females, but were more frequent in males at 250 and 500 mg/kg bw per day; they included increased quantities of protein, total bilirubin, cholesterol, inorganic phosphorus, potassium, and calcium, and γ -GT activity, while the concentration of glucose was decreased. Increased absolute and relative liver weights (> 40%) were seen in animals of each sex at 250 mg/kg bw per day and higher, and the relative kidney weights were increased (< 10%) in a dose-related fashion. There were no gross lesions at doses < 1000 mg/kg bw per day. Histopathological investigation showed kidney lesions (interstitial infiltration, tubular epithelial regeneration, and tubular dilatation) in males receiving 50 and 250 mg/kg bw per day and in animals of each sex at 500 mg/kg bw per day. The incidences of haemorrhage and oedema of the lung and hepatocellular swelling were increased at 500 mg/kg bw per day. A NOAEL was not identified (Naas, 1997).

Groups of 10 Sprague-Dawley rats of each sex, six weeks old at the beginning of the study, received ethoxyquin (purity, 97.6%) by gavage in corn oil at 0, 20, 40, 200, or 400 mg/kg bw per

day for 13 weeks. Minor overdosing (2–14%) of the group at 200 mg/kg bw per day on day 67 is considered not to have compromised the study. Ophthalmoscopy was performed before treatment and during week 12. A full post-mortem examination was performed on all animals, and samples of lung, liver, kidney, and gross lesions from all animals were examined histologically, as were more than 30 tissues from controls and from animals at the highest dose.

There were no deaths during the study. Clinical signs were seen in animals of each sex, but more often in females, at 200 and 400 mg/kg bw per day, including staining of various body parts and particularly the anogenital area, salivation, and brown urine. Body-weight gain was clearly reduced in males at 200 and 400 mg/kg bw per day, with a slight effect (10%) at 40 mg/kg bw per day; food consumption was similar in test and control groups. Haematological and clinical chemical parameters were altered in animals of each sex at 400 mg/kg bw per day, and many were also significant at 200 mg/kg bw per day. These included increased reticulocyte counts, total bilirubin, blood urea nitrogen, γ -GT activity, cholesterol, and thyroid-stimulating hormone; and decreased erythrocyte and leukocyte counts, prothrombin time, and glucose. Urine was more deeply coloured at 200 and 400 mg/kg bw per day, and the volume was increased in animals at the highest dose, with no change in specific gravity. There were no treatment-related effects on the eyes.

The main gross finding was reddened thyroids in animals of each sex at 200 and 400 mg/kg bw per day. The absolute weights of the liver and that relative to body weight were increased in a dose-related fashion (by 15–70%), and those of the kidneys increased by 4–20% in animals of each sex at 200 and 400 mg/kg bw per day; changes in the body-weight ratios of brain and testes are considered to be secondary to the reduced body weights. Histological examination identified the kidney as the main target organ in animals of each sex, with increased incidences of tubular mineralization, papillary necrosis, and cytoplasmic vacuolation in males at the high dose; and increased incidences of mineralization, papillary necrosis, and nephropathy in females at the high dose. The incidence of nephropathy was also increased in females at 200 mg/kg bw per day. The incidence of ultimobranchial cysts of the thyroid was increased in males receiving 200 and 400 mg/kg bw per day and females receiving 200 mg/kg bw per day. Increased incidences of cytoplasmic vacuolation of the adrenals, suppurative inflammation of the epididymides, non-suppurative inflammation of the prostate, mineralization of the lung, and alveolar histiocytosis were also seen in males at the high dose, and the incidences of inflammation of the oesophagus and epithelial hyperplasia of the thymus were increased in females at this dose. It should be noted that only gross lesions, liver, lung, and kidney were examined from groups at lower doses. As the decrease in body-weight gain in males at 40 mg/kg bw per day was part of a dose-response relationship and was not associated with reduced food consumption, the NOAEL for this study was 20 mg/kg bw per day (Naas, 1996a).

Dogs

A one-year study in three dogs given ethoxyquin by gavage was summarized in the 1969 monograph; the NOAEL was 3 mg/kg bw per day. The effects reported at the next highest dose (10 mg/kg bw per day) included renal nephrosis, increased bromosulphthalein retention indicating liver dysfunction, and abdominal tenderness (Hanzal, 1955; cited in 1969 JMPR monograph; Annex 1, reference 13).

Groups of one male and one female beagles received ethoxyquin (purity, 97.6%) by capsule at a dose of 0, 25, 50, 100, or 200 mg/kg bw per day for 28 days. All of the animals at 100 or 200 mg/kg bw per day group died or were sacrificed by day 17 or day 7, respectively; one female at 50 mg/kg bw per day was sacrificed on day 21. The signs seen in dogs that died and in survivors included hypoactivity, reduced defaecation, brown urine, and pale gums. Given the small initial group sizes and the deaths, only major, consistent changes are summarized here. Reduced body-weight gain and food consumption were seen at all doses. The serum activities of enzymes indicative of liver damage were increased at four weeks in all groups in which they were measured (25 and 50 mg/kg bw per day): alkaline phosphatase by fivefold, aspartate aminotransferase by threefold, alanine aminotransferase by 20-fold, and γ -GT by threefold; there were indications of

reduced activated partial thromboplastin times. The ratios of liver and kidney weights to body weight were increased at 25 and 50 mg/kg bw per day. Common post-mortem findings included redness of the gastrointestinal tract and darkened livers. Histological examination showed pigmentation of the liver in all treated animals but not in controls. A NOAEL was not identified (Naas, 1996b).

In a 90-day study, groups of five beagles of each sex were given ethoxyquin (purity, 97.6%) by capsule at 0, 2, 4, 20, or 40 mg/kg bw per day. Clear signs of toxicity were seen during the first seven weeks of the study at 40 mg/kg bw per day, including reduced body weight, staining of the body surface, brown urine, brown sclera, dark mucoid faeces, and emesis, and these groups received only empty capsules for the final six weeks of the study, effectively becoming reversibility groups. Investigations of clinical chemistry (including thyroid hormones), haematology, and ophthalmoscopy were performed before treatment and at weeks 4 and 12 or 13. Post-mortem investigations included microscopic examination of a wide range of tissues from all animals and special stains for pigment identification.

One female at the highest dose was sacrificed *in extremis* on day 13. Other findings were similar in males and females. Clinical signs including brown staining of the abdomen and urogenital area, brown urine, decreased faeces, and emesis were seen regularly at 20 and 40 mg/kg bw per day and occasionally during the 4 h after dosing at 4 mg/kg bw per day; these signs were still present between weeks 7 and 13 ('recovery') in animals at the highest dose. Body-weight loss occurred at 40 mg/kg bw per day in weeks 1–7, which reversed when dosing stopped, but females had a lower (12%) mean body weight than controls at termination. At 20 mg/kg bw per day, body-weight gain was reduced (60%) throughout the study. Food consumption was reduced at 20 (20%) and 40 mg/kg bw per day (up to 50%). The only notable change in haematological parameters was a dose-related decrease in activated partial thromboplastin times in males at 4 mg/kg bw per day and higher and in females at the highest dose. Marked increases in total bilirubin concentration and in alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, and γ -GT activities in serum, indicative of liver dysfunction, were seen at 20 mg/kg bw per day in weeks 4 and 12 or 13 and at 40 mg/kg bw per day in week 4 only; alanine aminotransferase and, to a lesser extent, alkaline phosphatase activities were also increased at 4 mg/kg bw per day. By week 13, the serum values in animals at 40 mg/kg bw per day (seven-week treatment, six-week recovery) had returned approximately to control values. There were no significant changes in absolute or relative organ weights. Treatment-related gross and microscopic findings were limited to the liver. At 20 and 40 mg/kg bw per day, a darkened appearance was associated microscopically with increased pigment deposition, hepatocellular necrosis, cytoplasmic vacuolation, and bile-duct hyperplasia; at 4 mg/kg bw per day, occasional findings of mild or moderate pigmentation, minimal hepatocellular necrosis, and vacuolation were recorded. The pigments were found to be porphyrin and bilirubin in most cases, some sections also staining for haemosiderin. The NOAEL was 2 mg/kg bw per day (Naas, 1996c).

(c) *Long-term studies of toxicity and carcinogenicity*

Mice

Solutions of ethoxyquin (as Santoquin®) at 10 or 50 mg/ml were administered subcutaneously to neonatal Swiss ICR/Ha mice on days 1 and 7 (0.1 ml) and 14 and 21 (0.2 ml) of age or as a single dose of 100 mg/ml (0.1 ml on day 1). Each dose was equal to 500, 2500, and 5000 mg/kg bw on day 1 and 250 and 1250 mg/kg bw on day 21. The groups consisted of 57 mice at the low dose, 53 at the intermediate dose, and 28 at the high dose. By the time the mice were weaned, 100% at the high dose, 74% at the intermediate dose, and 2% at the low dose had died; 15% of controls had died at this time. Small groups of mice were sacrificed at various times up to termination at week 53. A limited range of tissues and lesions were examined primarily for tumours. The incidences of pulmonary tumours and hepatomas were similar in the treated and control groups; a slight increase in the incidence of malignant lymphoma in four females at the low dose and two at the intermediate dose, with none in controls, was considered equivocal by the authors. The results indicates that four

subcutaneous administrations of ethoxyquin at near-lethal doses to neonatal mice did not significantly increase the incidence of tumours in mice up to one year of age (Epstein et al., 1970).

Rats

A two-year study of ethoxyquin in the diet of rats was summarized in the 1969 monograph. Groups of approximately 10 male and 10 female rats were fed diets containing ethoxyquin at concentrations of 0, 62, 125, 250, 500, 1000, 2000, or 4000 ppm for up to two years. The animals were sacrificed for autopsy after 200, 400, 600, or 715 days. The mortality rates were not significantly different from those of controls. Significantly reduced body-weight gain was seen at 2000 ppm after 225 days in males and after 21 days in females. After 200 days, increased relative liver and kidney weights were found in males at 250 ppm and in females at 1000 ppm. The haemoglobin values for animals of each sex were normal 100 and 300 days after the start of the experiment in rats fed 2000 and 4000 ppm. Histological changes in the renal cortex were clearly evident after 200 days in male rats receiving 2000 or 4000 ppm but not in females. All other organs were normal in animals of each sex after 200 days. After 400 days, lesions in the kidneys (pyelonephritis), liver, and thyroid were seen in males only. Similar lesions were seen for periods up to 717 days in animals of each sex, although they were more marked in males. Occasional tumours were found after 700 days, but the incidence was unrelated to dose, and they were also seen in controls. No clearly defined effects were evident after feeding 62 ppm, but minute lesions were present in the kidneys of two males receiving 500 ppm. It was difficult to distinguish the abnormalities in the group examined after 700 days from the pathological manifestations of senility after that time (Wilson & DeEds, 1959; cited in 1969 JMPR monograph; Annex 1, reference 13). The 1969 JMPR concluded that the NOAEL was 125 ppm, equivalent to 6 mg/kg bw per day. The small groups used in this study limit its sensitivity for detecting changes in rare events such as tumours with low background rates; however, the wide spread of doses and sequential sampling times provides a degree of assurance in the reported findings.

As part of an investigation of kidney and liver tumours induced by *N*-nitrosoethyl-*N*-hydroxyethylamine, one control group received only ethoxyquin. This group of 25 male Fischer 344 rats received a diet containing 8000 ppm ethoxyquin from nine weeks of age to termination at 41 weeks of age. Sections of liver, kidney, and gross lesions were investigated histologically. No γ -GT-positive foci, hyperplastic nodules, or hepatocellular carcinoma were found in the liver; no data were presented on kidney lesions. A control group of 25 male Fischer 344 rats received a diet containing 8000 ppm ethoxyquin as part of a parallel investigation of urinary bladder carcinogenesis induced by *N*-nitrosobutyl-*N*-hydroxybutylamine. After 32 weeks, there were higher incidences of simple hyperplasia and papillary or nodular hyperplasia of the urinary bladder than in groups receiving ascorbic acid or sodium erythorbate, the incidence of simple hyperplasia being greater than that induced by *N*-nitrosobutyl-*N*-hydroxybutylamine alone; no untreated controls were used. No urinary bladder papillomas or carcinomas were seen in the group receiving ethoxyquin only (Ito et al., 1985).

An almost identical study of urinary bladder carcinogenesis showed that 24 weeks' exposure to a diet containing 8000 ppm ethoxyquin, considered to be equivalent to 400 mg/kg bw per day, did not induce papillary or nodal hyperplasia or papilloma of the urinary bladder in a group of 15 male Fischer 344 rats (Miyata et al., 1985).

The dependence of the renal lesions produced by ethoxyquin on age and sex was investigated in Fischer 344 rats. Groups of four to eight male rats received diets containing ethoxyquin (purity, 90%) at 5000 ppm from three or eight weeks of age for 20, 26, or 30 weeks. Eight female rats received a diet containing 5000 ppm ethoxyquin for 30 weeks from eight weeks of age. Histopathological examination of the kidney comprised bromodeoxyuridine (BrdU) labelling, γ -GT histochemistry, haematoxylin and eosin staining, Alizarin red staining, and immunoblotting of urine samples for albumin and α_{2u} globulin. Body-weight gain was reduced by 10–15% in treated animals. In males, the absolute kidney weights were increased by 5–50%, with a

consequent increase in the relative weights; females showed a 12% increase in kidney:body weight ratios. Renal cortical changes were seen in all treated males, consisting of eosinic cytoplasmic inclusions in tubular epithelial cells and protein accumulation in the lamina of the tubules. In males dosed from three weeks of age, papillary necrosis, slight calcium deposition, and hyperplasia of the transitional epithelium of the renal pelvis were seen. The histological appearance of the kidneys of treated females was similar to that of controls, except for high concentrations of lipofuscin deposition. BrdU labelling was increased in males at 30 weeks, but not at 20 weeks, in both regenerating basophilic tubules and those staining normally with haematoxylin and eosin; BrdU labelling in females was not described. The concentrations of α_2 globulin in urine were slightly lower in treated males, but those of albumin were significantly increased. The time of first exposure can thus significantly alter the pattern of renal lesions in rats consuming diets containing ethoxyquin at 5000 ppm, equivalent to 250 mg/kg bw per day, exposure from three weeks of age resulting in papillary necrosis in addition to the cortical lesions seen in animals exposed from eight weeks of age (Manson et al., 1992).

Groups of 6–19 Fischer 344 rats of each sex, three weeks of age at the start of the study, received diets containing ethoxyquin (purity unspecified) at 0 or 5000 ppm for up to 18 months; one group received ethoxyquin in the diet for 24 weeks followed by 34 weeks on control diet. The study was designed to investigate the progression of renal lesions and involved interim sacrifices at 4, 12 or 14, 24, 58, and 78 weeks. The body-weight gain of treated females was reduced in weeks 1–5 and that of males from week 3 onwards; food consumption was reduced in animals of each sex during the first four weeks. Investigations of renal pathology showed a clear difference between males and females. Males had significant interstitial degeneration of the papilla at weeks 4 and 14, which progressed to necrosis with pyelonephritis of the cortex and urothelial hyperplasia of the renal pelvis by week 24. In females, interstitial degeneration of the papilla was only slight at week 14 and did not progress consistently. The chronic progressive nephropathy commonly seen in Fischer 344 rats was accelerated in animals receiving ethoxyquin. The authors reported that this was more marked in males, but the data presented do not substantiate that statement. A golden-brown pigmentation, found to be lipofuscin by Schmorl's stain, was noted in the proximal tubules of treated rats, particularly females. The lesions present at 24 weeks showed no evidence of reversibility after 34 weeks on control diets. The authors considered that there was no evidence for preneoplastic proliferative lesions. This study showed that ethoxyquin at 5000 ppm in the diet, equivalent to 250 mg/kg bw per day, is a potent nephrotoxin in young male Fischer 344 rats (Hard & Neal, 1992).

A number of reports have been published of the results of investigations into the effects of antioxidants, including ethoxyquin, on the induction of neoplasia and preneoplastic lesions by known carcinogens. The most comprehensive series of studies with ethoxyquin is probably that of Ito and co-workers (Ito et al., 1985; Miyata et al., 1985; Masui et al., 1986), who used Fischer 344 rats to investigate moderation of effects on the liver induced by *N*-nitrosodiethylamine, effects on the kidney and liver produced by *N*-nitrosoethyl-*N*-hydroxyethylamine, and effects on the bladder produced by *N*-nitrosobutyl-*N*-(4-hydroxybutyl)amine.

Groups of 18 six-week-old male Fischer 344 rats received an intraperitoneal injection of 200 mg/kg bw *N*-nitrosodiethylamine in 0.9% saline; two weeks later, they were transferred to a diet containing 0 or 8000 ppm ethoxyquin (purity unspecified) and underwent a partial (60%) hepatectomy one week later. The rats were sacrificed at week 8, and liver sections were stained with haematoxylin and eosin and a histochemical stain for γ -GT-positive foci. The rats receiving ethoxyquin had a significant ($p < 0.001$) decrease in the number of foci (0.9 versus 3.3/cm²) and in the area of the foci (0.06 versus 0.19 mm²/cm²) (Ito et al., 1985).

Groups of 23 or 27 six-week-old male Fischer 344 rats received drinking-water containing 0.1% *N*-nitrosoethyl-*N*-hydroxyethylamine for two weeks; between weeks 3 and sacrifice at week 32, they received diets containing 0 or 8000 ppm ethoxyquin (purity unspecified). All rats had γ -GT-positive foci, but the ethoxyquin-treated group had fewer (1 versus 21/cm²) and smaller (10 versus 22 mm²/cm²) foci. The group given ethoxyquin also had a reduced area of hyperplastic nodules (2.3 versus 7 mm²/cm²), and fewer animals had hepatocellular carcinomas (3/27 versus 11/23). Conversely, the kidneys of the ethoxyquin-treated group had higher frequencies of

atypical-cell foci (0.9 versus 0.2/cm²) and adenomas (5.6 versus 0.8/cm²), and there were more animals with foci (26/27 versus 12/23) and adenomas (17/27 versus 5/23) and larger foci (5.6 versus 0.9 x 10⁻² mm²/cm²) and adenomas (24 versus 8 x 10⁻² mm²/cm²) (Ito et al., 1985).

Groups of 25 six-week-old male Fischer 344 rats received drinking-water containing 0.05% *N*-nitrosobutyl-*N*-(4-hydroxybutyl)amine for four weeks; between weeks 4 and sacrifice at week 36, they received diets containing 0 or 8000 ppm ethoxyquin (purity unspecified). Examination of the urinary bladders showed that ethoxyquin had increased the incidence of animals with simple hyperplasia (25/25 versus 14/24), the incidence and extent of papillary or nodular hyperplasia (25/25 versus 8/24 and 9.4 versus 0.48/10 cm of basement membrane), the incidence and extent of papillomas (17/25 versus 5/24 and 1.1 versus 0.19/10 cm), and the incidence and extent of carcinomas (4/25 versus 1/24 and 0.17 versus 0.04/10 cm) (Ito et al., 1985). A similar study involving two weeks' dosing with *N*-nitrosobutyl-*N*-(4-hydroxybutyl)amine (0.05% in water) and sacrifice at 24 weeks also showed that dietary administration of ethoxyquin at 8000 ppm (purity unspecified) increased the incidence and extent of papillary or nodular hyperplasia but not of papillomas of the urinary bladder (Miyata et al., 1985).

This group of studies shows that ethoxyquin markedly reduces the preneoplastic effects of *N*-nitrosodiethylamine and *N*-nitrosoethyl-*N*-hydroxyethylamine on the liver, possibly by a combination of antioxidant effects and induction of detoxification mechanisms. The increase produced by ethoxyquin in the incidence of neoplastic and preneoplastic kidney lesions induced by *N*-nitrosoethyl-*N*-hydroxyethylamine may be secondary to the direct toxic effects of ethoxyquin on the kidney. The mechanism of the effects of ethoxyquin on *N*-nitrosobutyl-*N*-(4-hydroxybutyl)amine-induced urinary bladder neoplasia is unknown.

Dogs

Ethoxyquin was fed to two groups of 14 dogs and bitches at a dietary concentration of 0 or 300 ppm for five years. No effects were observed on haematological, urinary, or clinical chemical end-points (aspartate aminotransferase activity, blood urea nitrogen, and bromosulphthalein retention), organ weights, organ:body weight ratios, body weight, or gross or histopathological appearance (Monsanto, 1966; cited in 1969 JMPR monograph; Annex 1, reference 13). The 1969 JMPR concluded that the NOAEL in this study was 300 ppm, equivalent to 7.5 mg/kg bw per day.

(d) Genotoxicity

A number of published papers indicate that ethoxyquin is not genotoxic in bacterial systems (Table 5); however, these reports could not be validated as only minimal details were available. The results of assays in eukaryotic systems have not been reported.

Table 5. Results of assays for the genotoxicity of ethoxyquin

End-point	Test system	Concentration	Purity (%)	Result	Reference
Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	10–1000 µg/plate	'Pure'	Negative ^a	Joner (1977)
Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538; <i>E. coli</i> WP2 <i>hcr trp</i>	≥ 5000 µg/plate	NR	Negative ^a	Ohta et al. (1980)
Reverse mutation	NR	NR	NR	Negative	Zeiger (1993)
Gene mutation	<i>B. subtilis</i> H17 <i>rec*</i> and M45 <i>rec*</i>	0.2 ml	NR	Negative	Ohta et al. (1980)

^a With and without metabolic activation

(e) *Reproductive toxicity*

(i) *Multigeneration reproductive toxicity*

Rats

After 40 days on a diet slightly deficient in tocopherol and containing 0, 250, 500, or 1000 ppm ethoxyquin, rats were bred to produce three consecutive litters. The offspring of the first litter were used to produce a second-generation litter. The highest dose was discarded after production of one litter (reason unknown). No effects on reproduction, as reflected in fertility, litter size, or survival of offspring, were observed. The animals receiving the experimental diet produced young and raised them more successfully than controls, the 500 ppm diet being more effective than the 250 ppm diet (Wilson, 1956; Wilson & DeEds, 1959, cited in 1969 JMPR monograph; Annex 1, reference 13). The short dosing period before mating and the unknown purity and group size compromise this report to some extent, but it can be concluded that ethoxyquin in the diet at 500 ppm, equivalent to 25 mg/kg bw per day, has no marked effect on reproductive outcome.

Another study cited in the 1969 JMPR monograph had some results contrary to those described above. Groups of eight or nine female rats were placed on diets containing 0, 125, 375, or 1125 ppm ethoxyquin on the day of mating. The length of gestation was comparable in all groups, but the litter size was slightly depressed at doses of 375 ppm and higher, and at 1125 ppm the incidence of stillbirths was increased and survival to weaning was decreased. The NOAEL in this unusual study was 125 ppm, equivalent to 6 mg/kg bw per day. In a separate part of the same study, no effects were found on litter size, the number of stillbirths, survival to weaning, or weaning weight in rats receiving up to 1125 ppm ethoxyquin in the diet starting between days 1 and 10 of gestation (Derse, 1956; cited in 1969 JMPR monograph; Annex 1, reference 13).

Dogs

The effects of ethoxyquin on reproduction over two generations were studied in groups of beagles. Dogs were chosen as ethoxyquin is added to commercial dog food to help prevent oxidative deterioration. In the first mating (F_0), groups of five males and 10 females received diets containing ethoxyquin (Santoquin®) at a mean analytical concentration of 0, 100, or 225 ppm for a minimum of 82 days before pairing. The eight male and 13 female pups used subsequently for the F_1 matings received diets containing 0, 100, or 225 ppm ethoxyquin from weaning until breeding at 10–30 months (2nd estrus cycle in females). Semen samples were taken during the first week of treatment and at around the time of breeding in order to determine the volume, sperm count, motility, speed, and morphology. Animals were observed and underwent extensive physical examinations routinely; if possible, they were also observed during labour. Mating, whelping, and lactation indices were determined. Urine samples and blood samples were taken for haematology and clinical chemistry from fasted adults before treatment and at the end of the F_0 phase; at weeks 10, 23, 36, 49, and 62 and at termination in the F_1 growth phase; and at termination of the F_1 mating phase. Ophthalmological examinations were performed at the beginning and end of the F_1 growth and mating phases. All F_1 adults and pups that showed signs of toxicity were necropsied. A range of tissues from controls and F_1 adults at the high dose were examined histologically, with selected tissues from F_2 pups that showed clinical signs; the livers and gall-bladders from F_1 adults at the low dose and the adrenals and spleens from F_1 adult females at the low dose were also examined. Macroscopic and microscopic examinations were performed only on F_0 and F_1 animals that died or were sacrificed prematurely.

In the F_0 mating, there was considerable intra-group variation in body weights, but F_0 adults receiving 225 ppm ethoxyquin showed a trend for reduced body weight from the initiation of dosing to week 17 and during the latter stages of gestation. Males had reduced food consumption during most of the study. Two females at the high dose that were confirmed to be pregnant did not give birth. There were no other differences between the groups in mating performance, labour, birth, or weaning indices, semen parameters, or clinical signs. Litter size, pup survival, and pup weight and growth were similar in all groups. At 225 ppm, there was an increased number of pups of each sex with a raw or red anus, dehydration, nasal discharge, and excessive lachrymation; the

incidence of the last two signs was also increased at 100 ppm. Statistically significant increases in serum alkaline phosphatase activity were seen in male parents at the high dose and in female parents at the low and high dose; there was also an indication of reductions in monocytes and partial thromboplastin times in animals of each sex at the high dose, although all of the values were claimed to be within the normal ranges. There were no effects on urinary parameters. Remating of three female controls and two at 225 ppm from this phase which failed to mate during the initial phase was successful.

Among F_1 animals, one male at the low dose and two females at the high dose died or were sacrificed *in extremis*. The male was sacrificed because of suspected neurological signs; one of the females died of suspected heart disease, and the other was sacrificed because of pneumonia. The clinical signs included excessive lachrymation, dehydration, thinness, and pale gums and showed a dose-related increase in both the number of animals of each sex with a particular sign and the number of occasions on which it was observed. Males at the high dose had a lower mean body weight than controls up to week 48 of the study. Initially, animals at the high dose consumed more food than controls, but food consumption was consistently lower in weeks 8–18 in males and in weeks 8–30 in females. Considerable variations in haematological end-points were seen throughout the study in both treated and control animals. There was evidence of treatment-related effects on erythrocyte count, haematocrit, and haemoglobin, which were reduced by up to 11% relative to controls in treated males and females at weeks 10 and 23, and on partial thromboplastin times, which were reduced in females at the high dose in weeks 23 and 36 and in females at the low dose in weeks 23 and 62 and at the final analysis. Increased serum activities of alkaline phosphatase, γ -GT, and alanine aminotransferase and reduced albumin:globulin ratios were found in animals at the high dose in weeks 10, 23, and 36, with evidence of lesser perturbations at the lower dose. These changes are indicative of impaired liver function. The results of urinary analysis were unremarkable.

In the F_1 mating, there were no clear differences in semen analyses or mating, gestation, whelping, or weaning indices between control and ethoxyquin-treated animals. In adults, the only treatment-related clinical sign was excessive lachrymation, which occurred more frequently in males at the low and high doses than in controls. Haematological end-points were similar in all groups. Dose-related changes were seen in a number of clinical chemical parameters in females, which attained statistical significance ($p < 0.05$) at the high dose. These comprised reductions in glucose, cholesterol, protein, albumin, and albumin:globulin ratio, and increases in total bilirubin concentration and in γ -GT, alkaline phosphatase, and alanine aminotransferase activities. In males, the dose-related increases in alkaline phosphatase, γ -GT, and alanine aminotransferase activities did not attain statistical significance. Macroscopic examination showed dark-plum-coloured livers in one male and two females at the high dose and cervical lymph node haemorrhages in two females at the low and high doses; these lesions were possibly related to treatment as they were not present in control animals. Increases in the absolute weights of the spleen and testes and in the weights of these organs relative to the brain weight were seen in treated males, giving statistically significant increases in relation to body weight. In females, increases in the absolute and relative weights of the liver (10%), kidneys (10%), and spleen (40%) were reported but were not statistically significant. Histopathological examination showed that the liver, pituitary, and spleen were the target organs. The macroscopic finding of increased cervical lymph node haemorrhage in females was not confirmed. A dark-reddish-brown pigment, subsequently identified as protoporphyrin IX, was not found in the livers of controls or males at the low dose but was present in the livers of 7/13 females at the low dose, 2/7 males at the high dose, and 10/11 females at the high dose, with a dose-related increase in severity. The frequencies of fibrosis and haemorrhage of the spleen were increased in females at the high dose (3/11 versus 0/13 in controls), and the incidence of pituitary cysts was increased in animals at the high dose when compared with controls (2/6 versus 0/8 in males and 4/10 versus 2/12 in females).

Treated male pups had increased incidences of grey or pale gums, excessive lachrymation, and dehydration, and female pups had an increased incidence of dehydration. The pup weights at birth and to week 6 of gestation were slightly reduced ($< 10\%$), with a dose-related effect in female pups. An increased mortality rate in pups at the low dose was not seen at the high dose and was probably related to the larger litter sizes in this group; the rates of mortality were 7/62 controls, 24/91 at the low dose, and 10/77 at the high dose.

During the study, four males and one female at 100 ppm and two females at 225 ppm showed signs of neuropathy: The animals had impaired hindlimb function, inability to stand, and unsteadiness of the head and body which was found to be associated with myelin degeneration. Examination of clinically normal littermates showed no neurological deficits. The breeding records showed that all of the affected animals had a common male ancestor which was not in the breeding line of any of the control animals. When the parents of some of the affected pups were removed from the treated diets and mated, the incidence of neurologically affected animals was 17% in one litter and 25% in the other. The evidence from this part of the study is strongly indicative of a genetic etiology.

Estimation of the actual intakes of ethoxyquin in this study was confounded by up to twofold increased consumption during lactation and the fact that 180 and 360 ppm had to be added to obtain nominal concentrations of 150 and 300 ppm, however, the analysis showed mean initial values of 100 and 225 ppm. Although the actual amounts of food consumed varied during the study, a value of 25 g/kg bw per day was considered to be a representative mean, which resulted in intakes of 2.5 mg/kg bw per day ethoxyquin at 100 ppm and 6 mg/kg bw per day at 225 ppm.

The results of this study show that ethoxyquin in the diet at concentrations up to 225 ppm did not affect reproductive performance or outcome in beagles. There was no clear overall NOAEL because of increased incidences of clinical signs such as excess lachrymation and dehydration, clinical chemical changes and pigment deposition in the liver. The lowest dose, 100 ppm, equal to 2.5 mg/kg bw per day, was considered to be the minimal effect level (Gilman & Voss, 1995).

(ii) *Developmental toxicity*

Rats

In a range-finding study for teratogenicity in Sprague-Dawley rats, groups of eight mated females received ethoxyquin (purity, 97.6%) by gavage in corn oil at 0, 62, 125, 250, 500, or 1000 mg/kg bw per day on days 6–19 of gestation. All animals given 1000 mg/kg bw per day died or were sacrificed by day 9, and three animals given 500 mg/kg bw per day died between days 10 and 11 of gestation; post-mortem examinations did not show any adverse effects. Clinical signs of reduced defaecation, dark urine, and brown staining of fur were dose-related and affected all treated groups. Reduced food consumption and body-weight loss were seen at doses ≥ 125 mg/kg bw per day at the beginning of dosing; from day 9 onwards, body-weight gain was similar in all groups up to 500 mg/kg bw per day, and these animals had a 20% body-weight deficit by day 20 when compared with controls. Fetal weights were reduced at 500 mg/kg bw per day, but examination for external malformations, sex ratio, and crown–rump length showed no effects of treatment (Nemec, 1996a).

In the main study, groups of 25 mated female Sprague-Dawley rats received ethoxyquin (purity, 97.6%) by gavage in corn oil at 0, 50, 150 or 350 mg/kg bw per day on days 6–19 of gestation. The dams were sacrificed on day 20, their uteri and ovaries were examined, and all fetuses were investigated for weight, sex, and external and visceral malformations. The heads of one-half of the fetuses were examined by Wilson sectioning and the other half by mid-coronal section. All fetuses were stained with Alizarin Red S for skeletal investigation. There were no deaths during the study. Urogenital staining was seen in dams after treatment at the highest dose, and staining of other areas was also seen in these and in some animals receiving 150 mg/kg bw per day. At 350 mg/kg bw per day, dams lost weight on days 6–7, and a 13% reduction in body-weight gain compared with controls was evident on days 6–20; 150 mg/kg bw per day resulted in a 5% reduction in body-weight gain on days 6–20. Food consumption was reduced by 9% at 150 mg/kg bw per day and by 13% at 350 mg/kg bw per day. There were no significant findings in dams *post mortem*: uterine weights, litter size, resorptions, pre- and post-implantation losses, sex ratios, and fetal weights were similar in all groups. Isolated findings of malformations and anomalies were within the range in historical controls and showed no relationship to treatment. The overall incidence of variations was highest in controls, with no significant increase in any individual variation. The overall NOAEL for this study was 50 mg/kg bw per day on the basis of clinical signs (fur staining) and reduced maternal body weight at higher doses. The NOAEL for fetotoxicity was 350 mg/kg bw per day, the highest dose tested (Nemec, 1996b).

3. Observations in humans

Cases of dermatitis have been reported in workers handling fruit treated with ethoxyquin. A study with patch tests, cited in the 1969 monograph, showed that the cause was a sensitization reaction rather than direct irritation (Wood, 1965).

A number of reports (Burrows, 1975; van Hecke, 1977; Zachariae, 1978; Brandao, 1983) have indicated that ethoxyquin is the probable cause of an often severe dermatitis seen in workers who handle animal feed containing ethoxyquin. Positive results in patch tests have been recorded in affected workers given challenge concentrations of as little as 0.01% ethoxyquin in petrolatum (Zachariae, 1978). The authors of some of the reports indicated that airborne contamination and light sensitivity are implicated.

A study cited in the 1969 monograph indicated that no evidence of skin irritation or sensitivity had been reported in 20 years of ethoxyquin production (Kelly, 1960).

Comments

Published studies show that ethoxyquin is rapidly absorbed from the gastrointestinal tract of rats and mice, with peak blood levels within 1 h. Liver, kidney, and adipose tissue have the highest tissue concentrations. Excretion occurs predominantly via the urine and is rapid, with more than 85% of doses up to 25 mg/kg bw being excreted within 24 h. At 250 mg/kg bw, absorption and excretion are slowed, which is attributed to reduced gastric emptying, and only 50% of the dose is excreted within 24 h. Repeated oral doses of 25 mg/kg bw per day resulted in an excretion profile similar to that for single doses, but repeated administration of 250 mg/kg bw per day was reported to result in a profile similar to that for lower doses, indicating induction of metabolism, transport, and/or a return to normal gastric emptying. Biliary excretion and enterohepatic recirculation play a significant role in the toxicokinetics of ethoxyquin, more than 40% of an intravenous dose of 25 mg/kg bw being detected in the bile of bile-duct-cannulated rats. The metabolism of ethoxyquin involves *O*-deethylation or hydroxylation followed by conjugation as the sulfate or glucuronide. A proposed reaction scheme for the production of biliary metabolites involves epoxidation and the generation of reactive, electrophilic intermediates. No information on plant metabolites was available.

Ethoxyquin has low acute toxicity when administered orally ($LD_{50} = 1700$ mg/kg bw), dermally, or by inhalation. It is slightly irritating to the eyes and skin and had only very weak sensitizing potential when administered topically to guinea-pigs. Exposure to ethoxyquin in the workplace has been linked to allergic contact dermatitis, and the substance should be considered as a sensitizer in humans.

WHO has not classified ethoxyquin for acute toxicity.

The main target organ after repeated administration of ethoxyquin to rats for 28 days or more at doses of 50–1000 mg/kg bw per day was the kidney. Mechanistic studies with dietary concentrations equivalent to 250 mg/kg bw per day showed that the precise effects were dependent on the age at first exposure, were progressive, more severe in males than in females, and not reversible after 24 weeks of exposure. Other effects seen in rats exposed to ethoxyquin for 28 or 90 days at doses > 200 mg/kg bw per day were stained fur, brown urine, changes to haematological parameters, increased liver weights, and changes in clinical chemical parameters consistent with altered liver function. The overall NOAEL in the short-term studies in rats was 20 mg/kg bw per day.

In dogs given capsules containing ethoxyquin for 90 days at 0, 2, 4, 20, or 40 mg/kg bw per day, the liver was the primary target. Alterations in haematological parameters and clinical chemical changes indicative of altered liver function were seen at doses > 4 mg/kg bw per day, together with hepatocellular necrosis, vacuolation, and pigment deposition. Although staining indicated that the pigment was haemosiderin, a specific investigation showed it to be protoporphyrin IX. The overall NOAEL in short-term studies in dogs was 2 mg/kg bw per day. This is consistent with the results of an older, one-year study in dogs in which a NOAEL of 3 mg/kg bw per day was established on

the basis of findings suggestive of effects on the kidney and liver at 10 mg/kg bw per day.

No modern long-term studies of toxicity or carcinogenicity have been performed. In studies summarized by the 1969 Meeting in which ethoxyquin was administered to dogs at 0 or 300 ppm in the diet for 5 years or at 0, 3, 10, 50, or 100 mg/kg bw per day by gavage for one year, effects were observed in the liver and kidneys at doses of 10 mg/kg bw per day and above. The NOAEL was 300 ppm, equivalent to 7.5 mg/kg bw per day. A two-year study in rats that received dietary concentrations of 0, 62, 125, 250, 500, 1000, 2000, or 4000 ppm, published in 1959, gave no indication of carcinogenicity, with an overall NOAEL of 125 ppm, equivalent to 6 mg/kg bw per day; lesions in the kidney, liver, and thyroid gland were seen at higher doses. Mechanistic studies on tumour induction and promotion show that ethoxyquin induces both phase-I and phase-II xenobiotic metabolism. Although its incorporation into the diet at 8000 ppm after treatment with an *N*-nitrosamine reduced the formation of preneoplastic foci in the liver, it increased the incidence of preneoplastic and neoplastic events in the kidney and urinary bladder. No significant increase in tumour incidence was seen after one year in mice that received four subcutaneous, near-lethal doses of ethoxyquin.

Published reports of studies of bacterial mutagenicity indicate that ethoxyquin is not mutagenic in prokaryotic systems, but only limited details of the protocols and results were provided. No data were available on other genotoxic end-points.

No modern study of reproductive toxicity has been performed in rodents. Three studies in which rats received 0, 125, 250, 375, 500, 1000, or 1125 ppm in the diet, all with non-standard protocols, which were summarized by the 1969 Meeting, gave slightly contradictory results. Two of the studies, including the most extensive, apparently showed no effects on the aspects of reproduction investigated at doses up to 1125 ppm in the diet (equivalent to 56 mg/kg bw per day), while the other showed an increased incidence of stillbirths at 1125 ppm and decreased litter size at 375 ppm, with a NOAEL of 125 ppm (equivalent to 6 mg/kg bw per day).

A modern two-generation study of reproductive toxicity in dogs given diets containing 0, 100, or 225 ppm showed that ethoxyquin had no effects on reproductive parameters at 225 ppm (equivalent to 5.6 mg/kg bw per day), the highest dose tested. The clinical signs observed included dehydration, excess lachrymation, and evidence of hepatic toxicity, especially in bitches. The effects were seen at both doses and were consistent with the results of the short-term studies in dogs. The findings in bitches may have been related to increased consumption during gestation and lactation. The lowest dose tested, 100 ppm, equivalent to 2.5 mg/kg bw per day, was considered to be a minimal effect level.

A study of developmental toxicity in rats at 0, 50, 150, or 350 mg/kg bw per day showed that ethoxyquin is not fetotoxic or teratogenic at doses up to 350 mg/kg bw per day. Maternal toxicity, stained fur, and reduced body-weight gain were seen at 150 and 350 mg/kg bw per day. No studies of developmental toxicity have been performed in other species.

An ADI of 0–0.005 mg/kg bw per day was established on the basis of the minimal-effect level of 2.5 mg/kg bw per day in the multigeneration study in dogs and a 500-fold safety factor to account for the lack of a NOAEL in this study and for the incompleteness of the database. The multigeneration study of reproductive toxicity was of longer duration and more recent than a 90-day study in dogs treated by gavage with a NOAEL of 2 mg/kg bw per day.

An acute RfD was not allocated because ethoxyquin is of low acute toxicity. The Meeting concluded that the acute intake of residues is unlikely to present a risk to consumers.

Toxicological evaluation

Levels that cause no toxic effect

Rat:	125 ppm, equivalent to 6 mg/kg bw per day (two-year study of toxicity and carcinogenicity)
	500 ppm, equivalent to 25 mg/kg bw per day (two-generation study of reproductive toxicity)
	50 mg/kg bw per day (maternal toxicity in a study of developmental toxicity)
	350 mg/kg bw per day (developmental toxicity)

- Dog: 2 mg/kg bw per day (general toxicity in a 90-day study of toxicity)
 3 mg/kg bw per day (one-year study of toxicity)
 300 ppm, equivalent to 7.5 mg/kg bw per day (five-year study of toxicity)
 2.5 mg/kg bw per day (minimal effect level for general toxicity in a two-generation study of reproductive toxicity)
 5 mg/kg bw per day (reproductive performance; highest dose tested)

Estimate of acceptable daily intake for humans

0–0.005 mg/kg bw

Estimate of acute reference dose

Not allocated (unnecessary)

Studies that would provide information useful for continued evaluation of the compound

1. Studies of genotoxicity in mammalian systems
2. A long-term study of toxicity and carcinogenicity in rats that complies with modern guidelines
3. Observations in humans

List of end-points for setting guidance values for dietary and non-dietary exposure

Absorption, distribution, excretion and metabolism in mammals

Rate and extent of oral absorption	Rapid, > 50%
Dermal absorption	No relevant information
Distribution	Widely distributed; liver, kidney, adipose tissue
Potential for accumulation	Slight evidence of bioaccumulation
Rate and extent of excretion	> 85% eliminated within 24 h
Metabolism in animals	Extensive; no parent compound detected in urine
Toxicologically significant compounds (animals, plants and environment)	Metabolites considered of equivalent toxicity to parent compound

Acute toxicity

Rat :LD ₅₀ oral	1700 mg/kg bw
Rat: LD ₅₀ dermal	> 2000 mg/kg bw
Rat: LC ₅₀ inhalation	> 2.0 mg/L (whole-body exposure)
Skin irritation	Slightly irritating
Eye irritation	Slightly irritating
Skin sensitization	Sensitizing

Short-term toxicity

Target/critical effect	General toxicity in multigeneration study
Lowest relevant oral NOAEL	Dog: < 2.5 mg/kg bw per day (reproductive toxicity)
Lowest relevant dermal NOAEL	No data
Lowest relevant inhalation NOAEL	No data

Genotoxicity

No evidence of genotoxicity, but testing inadequate

Long-term toxicity and carcinogenicity

Target/critical effect:	Inadequate data
Lowest relevant NOAEL	Inadequate data
Carcinogenicity	No evidence of carcinogenicity, but testing inadequate

Reproductive toxicity

Reproduction target / critical effect	No adverse effect on reproduction
Lowest relevant reproductive NOAEL	Dog: 5 mg/kg per day, multigeneration study
Developmental target /critical effect	No adverse effect on development
Lowest relevant developmental NOAEL	Rat: 350 mg/kg/bw per day

<i>Neurotoxicity/Delayed neurotoxicity</i>	No data, but no concern from other studies		
<i>Other toxicological studies</i>	Not an initiator or promoter of liver tumours in rats Possible increase in urinary bladder preneoplastic and neoplastic changes		
<i>Medical data</i>	Contact allergic dermatitis reported in food handlers		
Summary	Value	Study	Safety factor
ADI	0–0.005 mg/kg bw	Dog, multigeneration study of reproductive toxicity	500
Acute reference dose	Not allocated (unnecessary)		

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