ETHOXYQUIN (addendum)

First draft prepared by
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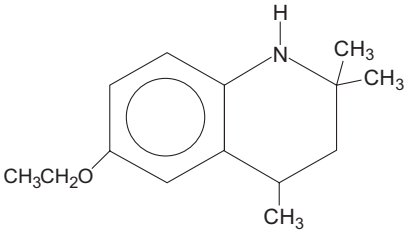
Explanation

Ethoxyquin is the International Office of Standardization (ISO) approved name for 1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline (Figure 1). It is used primarily as an antioxidant preservative in animal feed and dehydrated storage forage crops and as an antiscald agent in pears and apples. It is also used as a colour preservative in spices and as an anti-degradation agent in rubber.

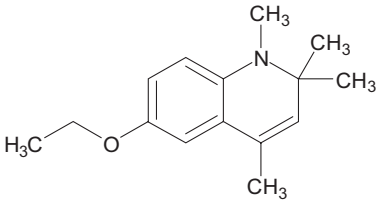
Ethoxyquin was first evaluated by the Meeting in 1969, when an acceptable daily intake (ADI) of 0–0.06 mg/kg bw was established based on the no-observed-adverse-effect level (NOAEL) in a long-term feeding study in dogs and a study of reproductive toxicity in rats. It was re-evaluated in 1998 within the periodic review programme of the Codex Committee on Pesticide Residues, and an ADI of 0–0.005 mg/kg bw was established on the basis of the minimal-effect level of 2.5 mg/kg bw per day for clinical signs and deposition of pigments in liver in a 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 1998 Meeting concluded there was no need to establish an acute reference dose (ARfD) for ethoxyquin. In 1999, the Joint Meeting reviewed the residue chemistry of ethoxyquin and concluded that the plant metabolites and degradation products, C–N and N–N dimers, demethylethoxyquin (DMEQ), methylethoxyquin (MEQ), dehydrodemethylethoxyquin (DHMEQ) and dihydroethoxyquin (DHEQ) were not formed in rats. In 2000, the Meeting recommended that information on the acute toxicity and genotoxicity of the plant metabolites/degradation products would be necessary to complete the evaluation of ethoxyquin.

The Meeting reviewed new data on the genotoxicity and acute toxicity of ethoxyquin and three of its plant metabolites/degradation products (MEQ, DHMEQ and DHEQ) in dogs, relevant

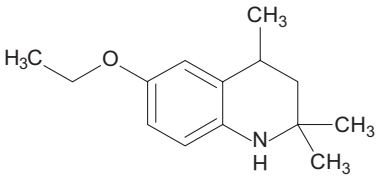
Figure 1. Chemical structures of ethoxyquin and three plant metabolites: MEQ, DHEQ and DHMEQ



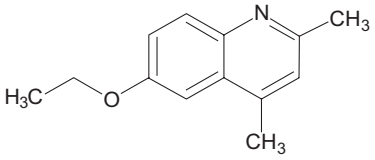
Ethoxyquin



Methylethoxyquin (MEQ)



Dihydroethoxyquin (DHEQ)



Dehydrodemethylethoxyquin (DHMEQ)

data from previous evaluations and other information from the published literature. DMEQ was not sufficiently stable to permit its synthesis and study.

All the new studies submitted for consideration at the present Meeting complied with good laboratory practice (GLP).

1. Toxicological studies

1.1 Acute toxicity in dogs

In this study, which complied with the principles of GLP, groups of six male and six female beagle dogs were given capsules containing ethoxyquin (purity, 98.93%) as a single oral dose at 50, 100 or 200 mg/kg bw. A concurrent control group received empty capsules on a comparable regimen. Four animals of each sex per group were scheduled for primary necropsy 24 h after dosing. The remaining two animals of each sex per group were assigned to a 14-day non-dosing recovery period.

The animals were observed twice daily for mortality and moribundity. Clinical examinations were performed daily and detailed physical examinations were performed weekly. Individual body weights were recorded daily. Food consumption was recorded daily and reported

weekly. Clinical pathology evaluations (haematology, serum chemistry and urine analysis) were performed before the initiation of dosing (study week −1) and during study weeks 0 (day after dosing) and 2 (recovery period). Ophthalmic examinations were performed before dosing (study week −1) and during study weeks 0 (all animals) and 2 (recovery group). Complete necropsies were performed on all dogs, and selected organs were weighed at the scheduled necropsies. Selected tissues from all animals were examined microscopically.

All animals survived to the scheduled necropsies. Body weights, food consumption and haematology parameters were unaffected by test article administration. No test article-related ophthalmoscopic findings were noted. There were no test article-related macroscopic findings or effects on organ weight parameters.

Higher mean serum alkaline phosphatase and alanine aminotransferase activities were noted in males at 50, 100 and 200 mg/kg bw and in females at 100 and 200 mg/kg bw when compared with the control group at the study week 2 recovery evaluation. However, it was noted that there were only two animals per group in this phase of the study. Higher total serum bilirubin concentrations were noted for both sexes in all groups and lower urea nitrogen concentrations were noted for females in all groups at the study day 1 evaluation (Table 1). In the absence of microscopic evidence of renal disease, lower urea nitrogen concentrations were attributed to slight hepatic insufficiency. The increased bilirubin noted at the study day 1 evaluation had returned to normal levels by the end of the recovery period (study week 2). A higher incidence of bilirubin in the urine as well as darker urine colour (amber-coloured) was noted at the study day 1 evaluation at all doses.

At the primary necropsy, microscopic findings were restricted to the liver and consisted of minimal to mild bile stasis for all dogs at 50, 100 and 200 mg/kg bw. Bile stasis was characterized by spherical accumulations of bile in intrahepatic bile canaliculi and was regarded as the microscopic correlate for increased total bilirubin concentration noted in serum chemistry profiles. In addition to bile stasis, deposits of hepatocellular glycogen were generally depleted in all dogs at 200 mg/kg bw. One male also displayed increased leukocytes in intrahepatic blood vessels and altered hepatocellular cytoplasm typified by a foamy to reticulated cytosolic morphology.

Test article-related microscopic findings observed at the recovery necropsy were restricted to the liver and consisted of minimal bile stasis in the liver in males at 50, 100 and 200 mg/kg bw and in females at 100 and 200 mg/kg bw.

Table 1. Summary of serum chemistry mean values in a study of acute oral toxicity in female dogs fed capsules containing ethoxyquin

Parameter	Time-point	*N*	Dose (mg/kg bw)			
			0 (control)	50	100	200
Urea nitrogen (mg/dl)	Week −1	6	13.0 ± 1.13	11.3 ± 2.17	11.0 ± 1.71	12.7 ± 2.68
	Week 0	6	15.5 ± 1.27	11.3* ± 2.65	11.1** ± 2.74	11.9* ± 2.0
	Week 2	2	14.8 ± 1.20	13.2 ± 0.07	10.4 ± 1.84	13.4 ± 1.41
Albumin : globulin ratio	Week 0	6	1.51 ± 0.111	1.73 ± 0.112	1.85* ± 0.249	1.84* ± 0.247
	Week 2	2	1.61 ± 0.255	1.74 ± 0.085	1.43 ± 0.071	1.54 ± 0.184
Total bilirubin (mg/dl)	Week −1	6	0.1 ± 0.05	0.1 ± 0.00	0.1 ± 0.04	0.1 ± 0.00
	Week 0	6	0.1 ± 0.04	0.3** ± 0.5	0.3** ± 0.08	0.5** ± 0.08
	Week 2	2	0.2 ± 0.07	0.2 ± 0.00	0.2 ± 0.00	0.2 ± 0.07

From Harriman (2004a)

* Significantly different from the control group at 0.05 using Dunnett's test

** Significantly different from the control group at 0.01 using Dunnett's test

The effects on serum biochemistry parameters indicative of effects on the liver observed at 50 mg/kg were minimal to mild. Their toxicological significance is equivocal. The Meeting did not consider that this was of toxicological significance and concluded that the NOAEL for ethoxyquin was 50 mg/kg bw in dogs (Harriman, 2004a).

1.2 Genotoxicity

The genotoxicity of ethoxyquin has been studied using in vitro and in vivo. The results are summarized in Table 2.

To test for antimutagenic activity with ethoxyquin in bone-marrow cells, three cytogenetic tests with distinct genetic end-points were applied. Cyclophosphamide, serving as the test mutagen, and ethoxyquin, the antioxidant, were administered consecutively by stomach tube to Chinese hamsters. While the dose of cyclophosphamide was the same in each test, the doses of ethoxyquin were increased up to a ratio of cyclophosphamide : ethoxyquin of 1 : 25, in some cases up to 1 : 50. The formation of sister chromatid exchanges induced by cyclophosphamide was not influenced by ethoxyquin—even at the highest dose. In the test for micronucleus formation; however, ethoxyquin drastically reduced the rate of micronucleus formation even at the lowest dose applied (20 mg/kg) and at a dose of 100 mg/kg abolished the effects caused by cyclophosphamide. This action of ethoxyquin against cyclophosphamide was also found in the rats and mice in this test system. Two inbred strains of mouse showed similar reactions, but the rate of micronucleus formation was higher and its decrease in response to increasing doses of ethoxyquin was more delayed. In the test for chromosome aberration, ethoxyquin also showed a distinct anticlastogenic response. At higher doses of ethoxyquin, all cyclophosphamide-induced chromosomal damage was reduced down to the level of spontaneous rates. The anticlastogenic effect of ethoxyquin was quantitatively similar in the tests for micronucleus formation and chromosome aberration. Only minor qualitative differences were recognizable (Renner, 1984).

The test for chromosomal aberration was employed to investigate the effect in vitro of a known antioxidant and food preservative, ethoxyquin (ethoxyquin,1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline) on human chromosomes. Lymphocytes obtained from three healthy donors were incubated with ethoxyquin at 0.01–0.5 mmol/l with and without metabolic activation. Stability studies using analysis by high-performance liquid chromatography (HPLC) showed that ethoxyquin was stable under the conditions of the lymphocyte cultures. The results of this assay showed that ethoxyquin induces chromosome aberrations—gaps and breaks as well as dicentrics and atypical translocation chromosomes (Blaszczyk et al., 2003).

Cytotoxicity was studied using ethoxyquin and its two salts, ethoxyquin hydrochloride (ethoxyquin-HCl) and ethoxyquin phosphate (ethoxyquin-P). It was shown that ethoxyquin was the most cytotoxic compound (IC_{50} = 0.09 mmol/l), while the lowest cytotoxic effect was observed for ethoxyquin-P (IC_{50} = 0.8 mmol/l). The properties of ethoxyquin and its salts were also analysed by terminal deoxynucleotidyl transferase-mediated dUTP nick end-labelling (TUNEL), which evaluates ability to induce apoptosis. Ethoxyquin induced apoptosis in cultured human lymphocytes, especially at concentrations of 0.25 and 0.5 mmol/l (Blaszczyk & Skolimowski, 2005).

Ethoxyquin was evaluated for genotoxicity in vitro and in vivo. There was no evidence of genotoxicity in tests for gene mutation in bacteria (*S. typhimurium* and *E. coli*). In Chinese hamster ovary cells, ethoxyquin induced chromosomal aberrations with and without metabolic activation, the effect being greater in the presence of an exogenous activating system. There was also an increase in polyploidy with and without metabolic activation. Increased endoreduplication was observed in the presence, but not the absence, of metabolic activation. In vivo, ethoxyquin gave negative results in a test for micronucleus induction in the bone marrow of mice. Although animals were treated at up to a maximum tolerated dose, there were no changes in the ratio of polychromatic to normochromatic erythrocytes (PCE : NCE) (Mecchi, 2004a).

The Meeting concluded that ethoxyquin does not exhibit genotoxic potential in vivo.

Table 2. Summary of results for genotoxicity with ethoxyquin

End-point	Test object	Concentration/dose	Purity (%)	Result	Reference
In vitro					
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	10.0–5000 µg per plate in DMSO	98.93	Negative[a]	Mecchi, (2004a)[b]
Reverse mutation	*E. coli* WP2 *uvrA*	33.3–5000 µg per plate in DMSO	98.93	Negative[a]	Mecchi, (2004a)[b]
Chromosomal aberration	Chinese hamster ovary cells	6.78–1000 µg/ml in DMSO ±S9	98.93	Significant increase in frequency of chromosomal aberrations at 20.0 and 30.0 µg/ml –S9 and at 15.0, 20.0, and 25.0 µg/ml +S9. Significant increase in frequency of polyploidy observed at 30.0 µg/ml –S9 and at 20.0 µg/ml +S9. Significant increase in endoreduplication observed at 10.0, 15.0, and 20.0 µg/ml +S9, but not –S9.	Murli (2004a)[b]
In vivo					
Micronucleus formation	CD-1 mice, six males per group, bone-marrow cells	Single oral doses of 375, 750, 1500 mg/kg bw in corn oil; sampling at 24 and 48 h	98.93	Negative	Erexson (2004a)[b]

DMSO, dimethyl sulfoxide; S9, 9000 × g supernatant from rodent liver

[a] With and without metabolic activation from

[b] Complied with GLP and statement of QA provided

1.3 Special studies: studies with plant metabolites

(a) Acute toxicity

In a study that complied with the principles of GLP, groups of six male and six female beagle dogs were given capsules containing one of three plant metabolites of ethoxyquin—MEQ (purity, 99.52%), DHEQ (purity, 99.52%) or DHMEQ (purity, 99.61%) as single doses at 50, 100 or 200 mg/kg bw. A concurrent control group received empty capsules in a comparable regimen. Four animals of each sex per group were scheduled for primary necropsy 24 h after dosing. The remaining two animals of each sex per group were assigned to a 14-day non-dosing recovery period.

The animals were observed twice daily for mortality and moribundity. Clinical examinations were performed daily and detailed physical examinations were performed weekly. Individual body weights were recorded daily. Food consumption was recorded daily and reported weekly. Clinical pathology evaluations (haematology, serum chemistry and urine analysis) were performed before the initiation of dosing (study week −1) and during study week 0 (day after dosing) and week 2 (recovery period). Ophthalmic examinations were performed before dosing (study week −1) and during study week 0 (all animals) and week 2 (recovery animals). Complete necropsies were performed on all dogs, and selected organs were weighed at the scheduled necropsies. Selected tissues were examined microscopically from all animals.

(i) MEQ

All animals survived to the scheduled necropsies. Body weight and food consumption were unaffected by test article administration. There were no test article-related effects on haematology parameters or ophthalmoscopic findings. There were no test article-related macroscopic findings or effects on organ weights. Test article-related clinical observations of emesis were noted in one or two females at 100 and 200 mg/kg 4 h after dosing as well as during the first 2 days of the recovery period.

Mean absolute neutrophil count in males at 100 mg/kg bw was significantly higher ($p < 0.05$) than the values for the control group on study day 1, but in the absence of any dose–response relationship this was not considered to be compound-related.

Higher total serum concentrations of bilirubin ($p < 0.05$ or $p < 0.01$) were observed in male and female dogs at doses of 50, 100 and 200 mg/kg bw, at the day 1 evaluation (Table 3). With the exception of one male at 200 mg/kg bw, the higher total bilirubin concentrations did not persist at week 2. Higher alanine aminotransferase, aspartate aminotransferase, gamma glutamyltransferase and alkaline phosphatase activities were noted in males and/or females at 50, 100 and 200 mg/kg bw at week 2 and were further indicative of the hepatic effects of the test article.

A higher incidence of bilirubin in the urine as well as a change in urine colour was noted at day 1. Urine was generally darker straw- to dark amber-coloured. Microscopically, minimal to moderate bile pigment accumulation in the canaliculi, hepatocytes and small bile ducts of the liver was noted in males and females at 50, 100 and 200 mg/kg bw at both the primary and recovery necropsies and corresponded to the higher serum bilirubin, alkaline phosphatase and gamma glutamyltransferase activities. In addition, giant multinucleated cells were noted, with a higher prevalence in the seminiferous tubules of the testes of males at 100 and 200 mg/kg bw at the primary and recovery necropsies.

The effects on serum biochemistry parameters indicative of effects on the liver observed at 50 mg/kg bw were minimal to mild. The Meeting did not consider that this was of toxicological significance and concluded that the NOAEL for MEQ was 50 mg/kg bw in dogs. The lowest-observed-adverse-effect level (LOAEL) was 100 mg/kg bw, the lowest dose tested (Harriman, 2004b).

(ii) DHEQ

All animals survived to the scheduled necropsies. Body weight, food consumption and haematology parameters were unaffected by test article administration. No test article-related ophthalmic findings were noted. There were no test article-related macroscopic or microscopic findings or effects on organ weights.

Test article-related higher incidences of clear material around the mouth and/or emesis were noted at 100 and 200 mg/kg bw in males and females at 4 h after dosing when compared with the control group. All the emesis occurred after the observation at 2 h. Three females in the group at 200 mg/kg bw were also observed to have clear discharge around the eye at 4 h after dosing.

Statistically significantly ($p < 0.05$ or $p < 0.01$) higher concentrations of total serum bilirubin and serum triglyceride (Table 4) were observed in females at 100 mg/kg bw and males and females at 200 mg/kg bw on day 1 compared with the control group values. Levels of these parameters in the recovery groups were not different from those in control animals. There was a higher incidence of bilirubin present in the urine of animals treated with DHEQ and the urine was generally darker in colour (dark amber-coloured) at the day 1 evaluation at all doses. The incidence of bilirubin in the urine was greater in females than in males. The liver appeared normal at all doses.

The effects observed at 50 mg/kg were minimal to mild, and their toxicological significance is equivocal. The presence of dark-coloured urine at the lowest doses was attributed to the presence of a chromophore in the compound or a derivative thereof. The Meeting did not consider

Table 3. Summary of serum chemistry mean values in a study of acute oral toxicity in female dogs fed capsules containing MEQ

Parameter	Time-point	*N*	Dose (mg/kg bw)			
			0 (control)	50	100	200
Total bilirubin (mg/dl)	Week –1	6	0.1 ± 0.00	0.1 ± 0.04	0.1 ± 0.00	0.1 ± 0.04
	Week 0	6	0.1 ± 0.04	0.3** ± 0.05	0.3** ± 0.04	0.4** ± 0.05
	Week 2	2	0.1 ± 0.00	0.1 ± 0.00	0.1 ± 0.00	0.1 ± 0.00

From Harriman (2004b)

MEQ, methylethoxyquin

** Significantly different from the control group at 0.01 using Dunnett's test

Table 4. Summary of serum chemistry mean values in a study of acute oral toxicity in female dogs fed capsules containing DHEQ

Parameter	Time-point	*N*	Dose (mg/kg bw)			
			0 (control)	50	100	200
Triglyceride (mg/dl)	Week –1	6	22 ± 3.9	29* ± 5.7	23 ± 3.6	28* ± 3.8
	Week 0	6	23 ± 5.3	31* ± 7.5	35** ± 3.1	37** ± 4.8
	Week 2	2	24 ± 7.1	22 ± 2.8	32 ± 0.0	32 ± 2.1
Total bilirubin (mg/dl)	Week –1	6	0.1 ± 0.00	0.1 ± 0.04	0.1 ± 0.04	0.1 ± 0.05
	Week 0	6	0.1 ± 0.00	0.2 ± 0.5	0.2* ± 0.08	0.3** ± 0.05
	Week 2	2	0.2 ± 0.07	0.2 ± 0.00	0.2 ± 0.07	0.2 ± 0.00

From Harriman (2004c)

DHEQ, dihydroethoxyquin

* Significantly different from the control group at 0.05 using Dunnett's test

** Significantly different from the control group at 0.01 using Dunnett's test

that this was toxicological significant and concluded that the NOAEL for DHEQ was 50 mg/kg bw (Harriman, 2004c).

(iii) DHMEQ

All animals survived to the scheduled necropsies. There were no test article-related effects on food consumption, haematology, serum chemistry or ophthalmic parameters. No test article-related macroscopic or microscopic findings were observed at the scheduled necropsies.

Clinical findings of clear material around the mouth and/ or emesis were noted sporadically at doses of 50, 100 and 200 mg/kg bw, and occasionally in untreated animals (Table 5). At higher doses (males and females at 100 and/or 200 mg/kg bw) the incidences were higher and more consistent, beginning as early as 30 min after dosing. In addition, five out of six males in the group receiving DHMEQ at a dose of 200 mg/kg bw had clear ocular discharge 4 h after dosing. These findings were considered to be related to administration of the test article at 100 and 200 mg/kg bw; however, the low incidence and absence of dose-dependency suggested that the effects at 50 mg/kg bw were incidental. Shivering was also noted for one female at 200 mg/kg bw at the time of dosing and at all time-points after dosing on study day 0, and 2 h after dosing for one female at 50 mg/kg bw and 4 h after dosing for two males at 200 mg/kg bw. The relationship of the latter findings to dosing with DHMEQ is unclear. Urine colour was darker in all treated groups on study day 1. In the absence of any other correlate, this was not considered as adverse. In three of six females at the highest dose, there was also a trace of bilirubin in the urine at this time. These changes did not persist during study week 2. There were no microscopic correlates.

Table 5. Summary of clinical findings (4 h after dosing) in a study of acute oral toxicity in male dogs given capsules containing DHMEQ

Area of body	Finding	Dose (mg/kg bw)			
		0 (control)	50	100	200
Behaviour/CNS	Shivering	0/0	0/0	0/0	2/2
Eyes/ears/nose	Clear discharge, left eye	0/0	1/1	1/1	2/2
	Clear discharge, right eye	0/0	0/0	1/1	5/5
Oral/dental	Wet clear material around mouth	1/1	0/0	2/2	2/2
	Emesis containing food	0/0	1/1	5/5	4/4
	Emesis containing white material	0/0	0/0	0/0	2/2
	Emesis containing yellow material	0/0	0/0	1/1	1/1

From Harriman (2004d)

CNS, central nervous system; DHMEQ, dehydrodemethylethoxyquin

Modest changes were observed in individual dose groups in mean values for erythrocyte volume fraction, percentage neutrophils, percentage lymphocytes and activity of alkaline phosphatase in the study week 0 evaluation. However, as there was no relationship with dose, these findings were considered to be incidental. On the basis of clinical signs, including the presence of clear material around the mouth and/ or emesis at higher doses, the NOAEL for acute oral toxicity of DHMEQ in dogs was 50 mg/kg bw (Harriman, 2004d).

(b) Genotoxicity

Three plant metabolites (MEQ, DHEQ, and DHMEQ) were evaluated for genotoxicity in vitro and in vivo. The results of these studies are summarized in Table 6. There was no evidence for genotoxicity of any of the metabolites in tests for gene mutation in bacteria (*S. typhimurium* and *E. coli*). In Chinese hamster ovary cells, MEQ and DHMEQ gave negative results in tests for clastogenic effects in the absence of exogenous activation, but both gave positive results in tests for chromosomal aberrations and endoreduplication in the presence of a metabolic activation system. DHEQ gave positive results for chromosomal aberrations with and without metabolic activation and for endoreduplication with metabolic activation.

In vivo, all three metabolites gave negative results in a test for micronucleus formation in the bone marrow of mice. MEQ and DHMEQ caused a reduction in the PCE : NCE ratio at the highest dose tested. DHEQ was not cytotoxic in bone marrow.

The Meeting concluded that none of the three plant metabolites tested, MEQ, DHEQ or DHMEQ, exhibited genotoxic potential in vivo.

Comments

Previous evaluations have established that ethoxyquin is rapidly absorbed from the gastrointestinal tract of rats and mice, with peak blood concentrations occurring within 1 h. The highest tissue concentrations were found in liver, kidney and adipose tissue. Excretion is predominantly as metabolites via the urine and is rapid, with > 85% of doses of up to 25 mg/kg bw being eliminated within 24 h. It has previously been reported that ethoxyquin has low acute toxicity when administered orally (median lethal dose, LD_{50} = 1700 mg/kg bw), dermally (LD_{50} > 2000 mg/kg bw) or by inhalation (median lethal concentration, LC_{50} > 2 mg/l) in rats.

Table 6. Results of studies of genotoxicity with three plant metabolites of ethoxyquin

(a) MEQ

End-point	Test object	Concentration/dose	Purity (%)	Results	Reference
In vitro					
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	3.33–5000 µg per plate in DMSO	99.52	Negative[a]	Mecchi (2004b)[c]
Reverse mutation	*E. coli* WP2 *uvrA*	33.3–5000 µg per plate in DMSO	99.52	Negative[a]	Mecchi (2004b)[c]
Chromosomal aberration	Chinese hamster ovary cells	5.43–800 µg/ml[a] in DMSO	99.52	Significant increase chromosomal aberration observed at 10.0 µg/ml[b], and in endoreduplication at 10.0 µg/ml[b]	Murli (2004b)[c]
In vivo					
Micronucleus formation	CD-1 mice, six males per group, bone-marrow cells	Single oral doses of 375, 750, 1500 mg/kg bw in corn oil; sampling at 48 h	99.52	Negative	Erexson (2004b)[c]

[a] With and without metabolic activation

[b] With metabolic activation

[c] Complied with GLP, statement of QA provided

(b) DHEQ

End-point	Test object	Concentration/dose	Purity (%)	Results	Reference
In vitro					
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	10.0–5000 µg per plate in DMSO	99.52	Negative[a]	Mecchi (2004c)[d]
Reverse mutation	*E. coli* WP2 *uvrA*	33.3–5000 µg per plate in DMSO	99.52	Negative[a]	Mecchi (2004c)[d]
Chromosomal aberration	Chinese hamster ovary cells	6.78–1000 µg/ml in DMSO[a]	99.52	Significant increase in endoreduplication observed at 125 µg/ml[c] and at 4.00 and 6.00 µg/ml[b]	Murli (2004c)[d]
In vivo					
Micronucleus formation	CD-1 mice, six males per group, bone-marrow cells	Single oral doses of 250, 500, 1000 mg/kg bw in corn oil; sampling at 24 and 48 h.	99.52	Negative	Erexson (2004c)[d]

[a] With and without metabolic activation

[b] With metabolic activation

[c] Without metabolic activation

[d] Complied with GLP, statement of QA provided

(c) DHMEQ

End-point	Test object	Concentration	Purity (%)	Results	Reference
In vitro					
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	3.33–2500 µg per plate in DMSO	99.61	Negative[a]	Mecchi (2004d)[c]
Reverse mutation	*E. coli* WP2 *uvrA*	10.0–3330 µg per plate in DMSO	99.61	Negative[a]	Mecchi (2004d)[c]
Chromosomal aberration	Chinese hamster ovary cells	5.43–800 µg/ml[a] in DMSO	99.61	Significant increase in chromosomal aberration observed at 25.0 and 50.0 µg/ml,[b] and in endoreduplication at 25.0, 100, and 150 µg/ml[b]	Murli (2004d)[c]
In vivo					
Micronucleus formation	CD-1 mice, 6 males per group, bone-marrow cells	Single oral doses of 500, 1000, 2000 mg/kg bw in corn oil; sampling at 24 and 48 h	99.61	Negative	Erexson (2004d)[c]

[a] With and without metabolic activation

[b] With metabolic activation

[c] Complied with GLP, statement of QA provided

In studies in dogs given ethoxyquin and three plant metabolites/degradation products (MEQ, DHMEQ and DHEQ) as single oral doses, the main target for all four compounds was the liver. Dogs were used in preference to rats as previous studies had shown that dogs are more sensitive to the toxic effects of ethoxyquin.

Dogs were fed capsules containing ethoxyquin, MEQ, DHMEQ or DHEQ as single doses at 50 to 200 mg/kg bw. Ethoxyquin, MEQ and DHEQ caused increases in serum and urinary concentrations of bilirubin at all doses. DHEQ had marginal effects on concentrations of bilirubin at the highest dose. Ethoxyquin and MEQ produced changes in the liver indicative of bile stasis and/or accumulation of bile pigment. Similar changes were reported previously in longer-term studies with ethoxyquin. During the 2-week recovery period (two dogs of each sex per group) elevations in serum enzymes for liver function (aspartate aminotransferase and alanine aminotransferase) were noted. After administration of the three metabolites/degradation products, clinical signs, including emesis and oral discharge, were noted. Based on the information available, the rank order of toxic potency for the four compounds (least toxic first) is: MEQ > ethoxyquin > DHEQ > DHMEQ. The effects observed at 50 mg/kg bw were minimal to mild, and their toxicological significance is equivocal. The presence of dark-coloured urine at the lowest dose of the compounds was attributed to the presence of a chromophore in the compound or a derivative thereof. The Meeting did not consider that this was toxicologically significant. The Meeting concluded that the NOAEL for all four compounds was 50 mg/kg bw.

Ethoxyquin and the three plant metabolites/degradation products were evaluated for genotoxicity in an adequate range of tests in vitro and in vivo. All compounds gave negative results in tests for mutagenicity in bacteria, with and without metabolic activation, confirming previous published reports on ethoxyquin. In a test for chromosomal aberrations in Chinese hamster ovary cells, all four compounds gave positive results. Ethoxyquin also gave positive results in a published study in which it was tested for chromosomal aberrations in isolated human peripheral blood lymphocytes. Although there have been positive findings for clastogenicity in vitro, all four compounds gave negative results in a test for micronucleus formation in bone-

marrow cells of mice in vivo. This confirms the results of a previous published study of macronucleus formation with ethoxyquin in bone marrow. It has also been reported in a published report that ethoxyquin gave negative results in tests for chromosomal aberrations and for sister chromatid exchange in vivo.

The Meeting concluded that ethoxyquin and the three plant metabolites/degradation products tested do not represent a genotoxic risk in vivo.

The 1969 and 1998 Meetings reviewed a number of published reports in which ethoxyquin had been administered to rodents for a prolonged period of time. These did not reveal any potential for ethoxyquin to produce a tumourigenic response.

In the absence of DNA reactivity and clastogenic effects in vivo and absence of tumours in rodents, the Meeting considered it unlikely that dietary exposures to this compound would pose any carcinogenic risk to humans.

The 1969 Meeting evaluated three studies of reproductive toxicity in which rats received diets containing ethoxyquin at concentrations of up to 1125 ppm. All had non-standard protocols, and the results were contradictory. Two of the studies, including the most extensive, showed no apparent effects on the end-points studied at up to the maximum concentration tested (equivalent to 56 mg/kg bw per day), while the other showed an increased incidence of stillbirths at 1126 ppm and decreased litter size at 375 ppm. The Meeting concluded that the design and reporting of these studies were inadequate.

The 1998 Meeting evaluated a two-generation study of reproductive toxicity in dogs given diets containing ethoxyquin at a concentration of 0, 100, or 225 ppm. There was no effect on reproductive parameters at up to the highest concentration tested (equivalent to 5.6 mg/kg bw per day). Clinical signs observed included dehydration and excess lachrymation. There was evidence of hepatic toxicity, particularly in the females. The effects were seen at 100 ppm, the lowest concentration tested, and were consistent with effects observed in short-term studies in dogs. The lowest concentration tested, 100 ppm (equivalent to 2.5 mg/kg bw per day) was considered to be a minimal-effect level for clinical signs of toxicity and liver effects.

A study of developmental toxicity in rats was evaluated by the 1998 JMPR. Rats were treated with ethoxyquin at doses of up to 350 mg/kg bw per day by gavage. Ethoxyquin was not fetotoxic or teratogenic at doses up to the highest tested. The NOAEL for maternal toxicity was 50 mg/kg bw per day on the basis of reduced body-weight gain at higher doses. No studies of developmental toxicity had been performed in other species.

Toxicological evaluation

The 1998 JMPR established an ADI of 0–0.005 mg/kg bw based on the minimal-effect level of 2.5 mg/kg bw per day for clinical signs in a multigeneration study in dogs and a safety factor of 500, because there was no NOAEL in this study and the database was incomplete owing to the lack of studies of genotoxicity and long-term studies of toxicity. No additional information was available to this Meeting on the long-term effects of ethoxyquin, although information on the genotoxicity of ethoxyquin and its three metabolites had been provided. The Meeting concluded that these compounds were not genotoxic in vivo. The acute toxicity of the plant metabolites/degradation products DHEQ and DHMEQ was no greater than that of ethoxyquin. The toxicity of the plant metabolite/degradation product MEQ appeared to be slightly greater than that of ethoxyquin. However, the Meeting concluded that a safety factor of 500 would be sufficient to allow for this difference in toxicity and confirmed the ADI established by the 1998 JMPR, extending it to cover the three plant metabolites/degradation products, MEQ, DHMEQ and DHEQ.

On the basis of the acute effects of ethoxyquin and its plant metabolites/degradation products in dogs, the Meeting established an ARfD of 0.5 mg/kg bw based on a NOAEL of 50 mg/kg bw for effects on the hepatic biliary system and clinical signs at higher doses from a study in dogs given single doses, and a safety factor of 100. The studies of reproductive toxicity in

rats were not considered to be an adequate basis for the derivation of an ARfD. The ARfD established applies to ethoxyquin and to the three plant metabolites/degradation products, MEQ, DHMEQ and DHEQ. It is applicable to the whole population.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Rat	Developmental toxicity[a]	Maternal toxicity	50 mg/kg bw per day	150 mg/kg bw per day
		Fetotoxicity	350 mg/kg bw per day[d]	—
Dog	1-year study of toxicity[a]	General toxicity	3 mg/kg bw per day	10 mg/kg bw per day
	Two-generation[b]	General toxicity	—	100 ppm, equivalent to 2.5 mg/kg bw per day[e]
		Reproductive performance	225 ppm, equivalent to 5.6 mg/kg bw per day[d]	—
	Single oral dose[c] study with parent and plant metabolites	Toxicity	50 mg/kg bw per day	100 mg/kg bw per day

[a] Gavage administration

[b] Dietary administration

[c] Capsule

[d] Highest dose tested

[e] Marginal effects on brain acetylcholinesterase activity, of equivocal toxicological relevance

Estimate of acceptable daily intake for humans

0–0.005 mg/kg bw, applicable to ethoxyquin, MEQ, DHMEQ and DHEQ

Estimate of acute reference dose

0.5 mg/kg bw, applicable to ethoxyquin, MEQ, DHMEQ and DHEQ

Information that would be useful for the continued evaluation of the compound

Results from epidemiological, occupational health and other such observational studies of human exposures.

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