OLAQUINDOX

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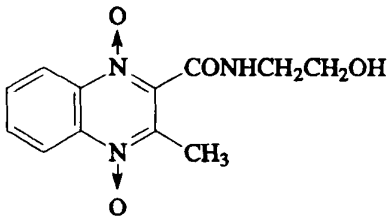
IDENTITY

Chemical name: N-(2-Hydroxyethyl)-3-methyl-2-quinoxalinecarboxamide-1,4-dioxide

Structural formula:



Molecular formula: $C_{12}H_{13}N_3O_4$

Molecular weight: 263.25

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient:

Appearance: Pale yellow crystals

Melting point: dec. 209°

Solubility: Slightly soluble in water, insoluble in most organic solvents
pKb = 11.3 at 25°, pH = 7.3 (in water)

SUMMARY OF THE EVALUATION AT THE 36TH MEETING OF JECFA (1990)

Pharmacokinetics

Two studies using 3-position ^{14}C-radiolabeled olaquindox were reported by the sponsors. In the first study (Duhm, et al., 1973, Bayer Pharma Report no. 4151) five female pigs were administered 2 mg/kg bw ^{14}C-olaquindox directly into the duodenum using physiological saline solution. More than 90% of the radioactivity was excreted in the urine within 24 hours and 5% of the radioactivity was extreted in the feces, most within the first 48 hours. The one male pig in the second study was given 2 mg/kg radiolabeled drug in a gelatine capsule by means of a stomach tube (Maul, Seng, and Wendisch, 1979, Bayer Pharma Report no. 8114). 95% of the radioactivity was excreted in the urine over the 48-hour collection period.

In the first study, the radioactivity in the plasma reached the maximum value, equal to 2 mg/l of olaquindox equivalents, one hour after dosing. There was a rapid decline in concentration to 5 μg/kg in about 48 hours with a plasma half life of four hours. In one pig where measurements were continued for 28 days, the half life for the plasma radioactivity after the first 48 hours was nine days. Based on radioactivity measurements, the concentration of olaquindox equivalents in the plasma at 28 days was about 1 μg/kg. The halflife of the residues is similar in liver, kidney, muscle and adrenals and has a value of about five days.

Metabolism in Pigs

Olaquindox metabolism was examined in the urine in two studies using ^{14}C-radiolabeled olaquindox. The first study (Duhm, et al., 1973, Bayer Pharma Report no. 4151) used five female pigs dosed with C-3 radiolabeled drug and the second study (Maul, Seng and Wendisch, 1979, Bayer Pharma Report no. 8114) used one male pig dosed with olaquindox uniformly labelled in the phenyl ring. The results of both studies are similar.

Olaquindox accounts for more than 60% of the original dose in excreted products. Four other metabolites were identified and made up most of the remaining residues in urine. Olaquindox is metabolized either by oxidation of the alcohol to the carboxylic acid on the side chain at position 2 on the quinoxaline ring and/or reduction of one or both of the N-oxide groups at positions 1 and 4. No mechanism of metabolism is provided.

In the first study, urine was collected at 1.5, 3, 5, and 24 hours after dosing the female pigs. The metabolic profile in urine was determined by a two-dimensional separation using high voltage electrophoresis and paper chromatography. Five metabolites were found. The major product (about 70% of the radioactivity) was parent drug and the second major product (about 16% of the radioactivity) was a mono-N-oxide of olaquindox. The three other metabolites were quantified but not identified. In the second study using one male pig, the residues were examined in the tissues at 48 hours and in plasma at 2, 7, 24, and 48 hours post dosing. The metabolites were separated by a combination of thin layer chromatography and high voltage electrophoresis. The metabolites were identified by autoradiography and comparison with authentic standards. The metabolites identified are shown in Table 1.

Table 1. Metabolite Profile of Olaquindox in Male Pig Urine

Metabolite	Side Chain at the 2 Position	Position of -N-oxide	% Total ^{14}C
I (parent)	-CO-NH-CH_2-CH_2-OH	1 and 4	65
II	-CO-NH-CH_2-CH_2-OH	4	7
III	-CO-NH-CH_2-COOH	1 and 4	3
IV	-CO-NH-CH_2-COOH	4	6
V	-CO-NH-CH_2-COOH	1	1
VI	-CO-NH-CH_2-COOH	None	< 1

(I and II were also identified in female pig urine).

A fraction of the urine was hydrolysed with the enzymes glucuronidase and sulfatase but subsequent analysis showed that there was no evidence of conjugated metabolites.

Plasma concentrations of radioactive compounds were low in all samples and it was only possible to identify metabolites in the samples collected at two and seven hours after dosing. Two dimensional thin layer chromatography was used to separate and identify the metabolites. The measurements were made close to the limits of detection and only parent drug (I) and metabolite II were definitely identified. Metabolite IV was probably present in the seven hour plasma sample.

Muscle, liver and kidney samples were collected at sacrifice 48 hours after dosing. It was not possible to identify the metabolites because concentrations were below the sensitivity of the method. No information is provided on the metabolic profile in edible tissues, although the levels of total radioactivity are recorded.

Five residue depletion studies were reported in the original assessment of olaquindox to identify and characterize the residues. The methods used for these studies were thin layer chromatography, high performance liquid chromatography and a combination of thin layer chromatography and gas liquid chromatography. All studies used doses that were 0.2 to 5 times the recommended feeding treatment. It was not possible, however, to measure residues in tissues at withdrawal times longer than 48 hours because the analytical methods were not sensitive enough.

No bioavailability studies were reported by the drug sponsor. Based on the high recovery rate (>95% recovery of radioactivity in 48 hours), and the fact that the residues contain olaquindox as a major component together with several closely related metabolites, there is good reason to believe that almost all of the residues are bioavailable.

In the final appraisal, no MRL was recommended until a tissue depletion study has been carried out to characterize the nature and amount of the residues in tissues and identify a suitable marker compound.

1993 DATA SUPPLEMENT

Total Residue Depletion in Pigs

Four additional total residue depletion studies were conducted on pigs using olaquindox, uniformly labelled ^{14}C in the phenyl ring. Three of the four studies (Ackerman, et al., 1977, Analytical Development Corp., Unpublished BAYVET Report no. 54625; Wilkes, Tapprich and Wargo, 1978, Analytical Development Corp., Unpublished BAYVET Report no. 70277; Waggoner, 1990, Unpublished MOBAY Report no. 74001) measured radioactive total residues after a single daily oral administration of 5 mg/kg body weight ^{14}C-olaquindox for seven consecutive days and covered a zero (two hour) to 50 day withdrawal period. The 5 mg/kg body weight dose corresponded to a feed inclusion of about 100 mg/kg (double the recommended) dose for growth promotion. Table 2 summarizes the residue findings of these high dose studies.

Table 2. Total Olaquindox Related Residues in Pigs Using a 5 mg/kg Dose (^{14}C-Olaquindox Equivalents in $\mu g/kg$)

No. Animals	Time	Blood	Muscle	Fat	Liver	Kidney
1M, 1F	2h	3350	2935	1295	4085	11145
1M, 1F	7d	30	41	12.5	154	108.5
2M, 1F	14d	23.7	21.7	5.7	59.7	49.3
2M, 1F	28d	10.6	8	2.7	13.3	11.3
1M, 2F	40d	-	2.4	7.7	3.4	2.6
2M, 1F	50d	-	1.6	<5	2.0	1.1

Semilogarithmic plots show excellent linear depletion in muscle, liver and kidney. The regression curves (y in $\mu g/kg$, x in days) are:

Muscle: $\log y = -0.03261x + 1.78588, \quad r^2 = 0.9913$

Liver: $\log y = -0.04197x + 2.32075, \quad r^2 = 1.0000$

Kidney: $\log y = -0.04963x + 2.40668, \quad r^2 = 0.9994$

The fourth study (Figge, 1982, NATEC, Unpublished BAYVET Report no. 72488) used a 2.5 mg/kg body weight per day oral dose of phenyl ring uniformly radiolabeled ^{14}C-olaquindox. The drug was administered in two 1.25 mg/kg doses daily 12 hours apart for five consecutive days (10 doses). The 2.5 mg/kg daily oral dose corresponded to a feed inclusion of about 50 mg/kg, the recommended dose, for growth promotion. Table 3 summarizes the residue findings.

Table 3. Total Olaquindox Related Residues in Pigs Using a 2.5 mg/kg Dose (^{14}C-Olaquindox Equivalents in μg/kg)

No Animals	Time	Blood	Muscle	Fat	Liver	Kidney
1M, 2F	2h	560	503	356	996	2399
1M, 1F	2d	63.4	34.2	25.7	289.6	192.7
1M, 1F	7d	42.2	21.0	17.1	90.2	67.1
2M, 2F	14d	51.6	11.6	2.4	31.7	23.9
3M, 3F	28d	6	4.1	0.9	8.2	6.7

There is a clear dose dependence of the residues. Table 4 summarizes the residues after 14 and 28 day withdrawal for the 5 mg/kg and the 2.5 mg/kg dose studies in pigs.

Table 4. Olaquindox Related Residues in μg/kg for 5 mg/kg and 2.5 mg/kg per day Dosage in Pigs

Withdrawal Time	Dose mg/kg/day	Muscle μg/kg	Liver μg/kg	Kidney μg/kg	Fat μg/kg
14	2.5	11.6	31.7	23.9	2.4
	5	21.7	59.7	49.3	5.7
28	2.5	4.1	8.2	6.7	0.9
	5	8.0	13.3	11.3	2.7

Assessing these four studies, olaquindox residues were proportional to the administered dose and these residues depleted from all measured tissues at a constant rate with time, with no apparent bound residues in tissue. At the recommended dose administration (2.5 mg/kg/day) and a 28 day withdrawal period, total residues in muscle were less than 5 μg/kg and less than 10 μg/kg in liver and kidney.

IDENTIFICATION OF METABOLITES

Blood

Identification of metabolites of olaquindox in urine of treated pigs has been summarized in Table 1. In a later study, Maul, Seng and Wendisch (1979, Unpublished BAYER Pharma Report no. 8725) used a single 2 mg/kg dose of radiolabeled olaquindox and withdrawal times of 2 and 7 hours. Besides the parent drug (I, see Table 1), two mono-N-oxides could be identified in blood plasma (II and IV, see Table 1). None of the blood plasma residues could be identified at 24 and 48 hours after dosing. Organ tissues 48 hours after dosing did not contain sufficient radioactivity for identification of individual metabolite components. Results are summarized in Table 5.

Table 5. Olaquindox Related Residues in Blood Plasma

Withdrawal Time (hrs)	^{14}C-Olaquindox Residues (μg/kg)	No. of Substances Detected	Identified Structures (%)
2	0.071	5	I (43), II (12)
7	1.000	9	I (52) II (9) IV (2)
24	0.034	5	-
48	0.006	1	-

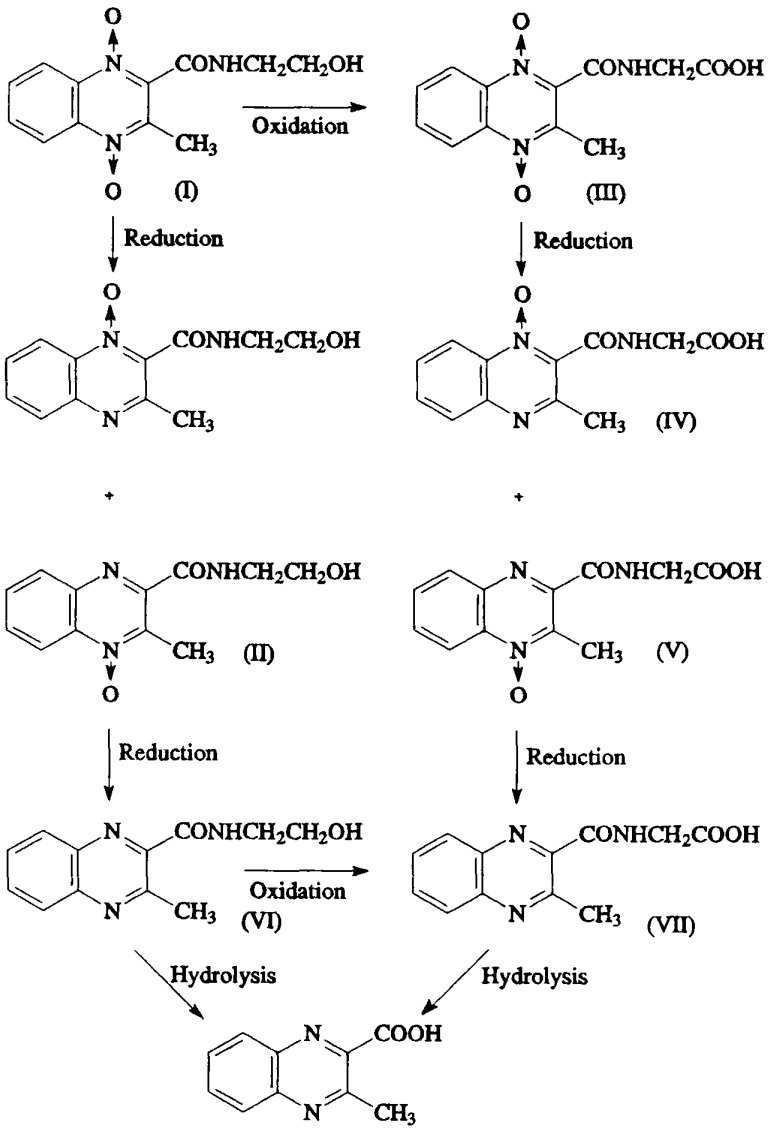
Tissue

Using a 5 mg/kg body weight ^{14}C-radiolabeled olaquindox dose per day for seven days, identification of metabolites for liver and kidney was studied. Pigs were sacrificed two hours after the last dose. A total of 79% of the total residue was recovered from liver and 93% from kidney. For both liver and kidney, more than 80% of the extracted residue migrated at a single spot that was identified by mass spectrometric analysis to be the bis-N-oxide reduced parent compound 2-[N-(2-hydroxyethyl)-carbamoyl)]-3-methylquinoxaline by comparison to a reference standard (Anonymous, 1977, Analytical Development Corp., BAYVET Report no. 54959.) This compound was subsequently isolated from liver tissue and its structure confirmed in a feeding study with non-radiolabeled drug as well as a ^{14}C-radiolabeled drug study (Gau, Ploschke and Wuensche, 1983, Unpublished BAYER Pharma Report no. 11998). Identification was verified by preparative HPLC and mass spectrometry. After five days dosing and sacrifice two hours after the last dose, 60% of the olaquindox radioactivity in the liver consisted of this single compound.

A second metabolite isolated in liver and representing 13% of the total residues in liver after five days dosing and a two hour withdrawal period was identified by a reverse isotope dilution technique, NMR and mass spectrometry analysis, as the carboxylic acid derivative of the reduced parent compound, 2-carboxymethylaminocarbonyl-3-methylquinoxaline (Maul, 1983, Unpublished BAYER Pharma Report no. 72827). Virtually no bound residues could be detected in the organ tissues.

The proposed metabolic pathway consists of a series of reduction and oxidation steps and hydrolysis as presented in Figure 1. The 3-methylquinoxaline-2-carboxylic acid (MQCA) compound has been identified by the sponsor as the residue marker for residues of olaquindox. This conclusion is based on studies referenced above and analysis of tissues following two longterm studies using unlabeled olaquindox in pigs at 25 and 100 mg/kg in feed.

Figure 1. **Olaquindox - Metabolic Pathways**



METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

A number of residue methods were reported for olaquindox residues in pig tissue and have been summarized in the previous report (Anonymous, 1990, FAO Food and Nutrition Paper 41/3). Numerous attempts have been made to improve the methods. However, residues were almost nondetectable later than 48 hours after the last drug treatment. Based on studies reported in the submission by the sponsor and consideration of the metabolism of olaquindox and related compounds (e.g., carbadox) it was concluded that 3-methylquinoxaline-2-carboxylic acid (MQCA, see Figure 1) was the proposed residue marker for olaquindox. Based on residue data summarized in Tables 2 and 3, liver had the highest tissue residue concentration up to 28 days withdrawal. Considering the analytical difficulties with liver tissue, however, it was concluded by the sponsor that muscle would be the suitable target tissue for residue analysis and regulatory control.

A new residue method quantitates the marker compound (MQCA) at 1 μg/kg in swine muscle (Waggoner and Kohlenberg, 1990, Unpublished MOBAY Report no. 74072). The method employs GC/MS to identify and quantitate the methyl ester of MQCA by comparison with a ^{13}C-MQCA internal standard. The response ratio of MQCA internal standard to MQCA was linear from 0.5 to 10 μg/kg. Recoveries of MQCA from muscle fortified with known amounts of analytical standard were 90-138% (average 103%) for the same concentration limits. The method is reported to be quantitative at concentrations below 0.5 μg/kg. The sponsor states that radio validation data indicate that the marker compound represents approximately 25% of the total incurred residues of olaquindox.

No other methods have been published for MQCA residue analysis at the low μg/kg concentration. Method sensitivity for residues of olaquindox, measured as parent drug, is limited to 20 μg/kg.

TISSUE RESIDUE DEPLETION STUDIES

Two residue studies have been reported using the method referenced above. In one study feeder pigs were dosed using medicated feed at 25 mg/kg for 28 days (Waggoner and Kohlenberg, 1990, Unpublished MOBAY Report no. 74072). Muscle samples were analyzed for MQCA residues. The depletion of MQCA was linear over the 8-20 day withdrawal period. In a similar study, pigs were dosed with medicated feed at 100 mg/kg for 68 days. (Waggoner and Kohlenberg, 1990, Unpublished MOBAY Report no. 74043). Steady state concentration of residues was reached at 29 days after the start of medication. Animals were sacrificed at specified intervals following medicated feed treatment. Depletion of residues was linear. Results of these two feeding studies are tabulated in Table 6 and Table 7, respectively.

Table 6. Residues of MQCA-Marker in Pig Muscle After Treatment with 25 mg/kg Olaquindox in Feed

Withdrawal Time (Days)	Number of Animals	Concentration MQCA in μg/kg (±SD)
0	4	53.2 (14.4)
8	4	3.1 (0.8)
13	4	2.0 (0.5)
16	4	1.0 (0.5)
20	4	0.9 (0.5)

Depletion of MQCA residues was linear over the 8-20 day withdrawal period, as noted by the following equation:

$$\log y = -0.051876x + 1.88717, \qquad r^2 = 0.8736$$

$$y = \mu g/kg \text{ (ppb)}; \; x = \text{days}$$

Table 7. Residues of MQCA Marker in Pig Muscle After Treatment with 100 mg/kg Olaquindox in Feed

Withdrawal Time (Days)	Number of Animals	Concentration MQCA in μg/kg ($\pm$SD)
7	4	16.4 (1.4)
10	4	9.7 (1.3)
14	4	8.7 (0.2)
17	4	8.8 (1.1)
25	4	3.9 (0.7)
36	5	3.0 (0.6)

Depletion of residues was also linear at the higher dose level. The regression equation is

$$\log y = -0.02452x + 2.30436, \qquad r^2 = 0.9607$$

$$y = \mu g/kg \text{ (ppb)}; \; x = \text{days}$$

In summary, the GC/MS residue method is capable of detecting and quantitating the olaquindox metabolite MQCA at 1 ug/kg and below. Residue studies with olaquindox medicated feed at 25 and 100 mg/kg show that the depletion of residues is reasonably linear and that residues in muscle tissue deplete to less than 5 μg/kg after 8 days at the low dose feeding level and after 25 days at the high dose feeding level.

APPRAISAL

This is the second time olaquindox has been reviewed by the Committee. New data has been provided by the sponsor to address concerns from the 36th meeting of the JECFA regarding characterization of metabolites and residues in tissues, identification of marker residue and a more sensitive analytical method to detect residues at long withdrawal periods. Results are noted below.

Total ^{14}C- and MQCA-residues tend to deplete at comparable rates and in an approximately linear manner although there are no studies using the same dose levels to verify this relationship. Residues of MQCA in muscle are estimated to account for approximately 25% of the total incurred residues based on radiolabeled studies with withdrawal times of 8-20 days. Studies furthermore show that greater than 95% of the radioactivity from dosed animals is excreted in urine and faeces within 48 hours and there is no evidence of bound residues attributed to residues of olaquindox.

The GC/MS analytical method developed by the sponsor (using an ^{13}C-radiolabeled internal standard) is

sufficiently sensitive to quantitate MQCA residues in muscle and liver tissue at level of 1 μg/kg, and confirm the identity of proposed marker compound.

Thus, MQCA would be suitable as the marker residue provided that an MRL for olaquindox was recommended at or above 1 μg/kg as MQCA or 4 μg/kg total residues of olaquindox in muscle tissue. Regarding analysis of liver tissue, the sponsors do acknowledge that the liver is not an easy tissue to analyze, but that the method is equally suitable with the same analytical sensitivity.

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