Date of Approval: January 30, 1998

CORRECTED FREEDOM OF INFORMATION SUMMARY

SUPPLEMENT TO NADA 041-061

I. GENERAL INFORMATION

NADA Number:	041-061
Sponsor:	Pfizer, Inc., 235 East 42d St., New York, NY 10017
Established Name:	carbadox
Trade Name:	MECADOX® 10 Type A medicated article
Marketing Status:	Over-the-counter (OTC)
Effect of Supplement:	This supplement provides for the codification of a revised tolerance for residues of carbadox in edible tissues.

II. INDICATIONS FOR USE

MECADOX® 10 is indicated for the control of dysentery and bacterial enteritis and for growth promotion in swine.

III. DOSAGE FORM, ROUTE OF ADMINISTRATION, AND DOSAGE

A. Dosage Form

MECADOX® 10 is a Type A medicated article used in the preparation of finished Type C medicated feeds for swine.

B. Route of Administration

MECADOX® 10 is administered orally in feed.

C. Recommended Dosage

Carbadox is administered *ad libitum* in a final feed at a concentration of 55 ppm. Medicated feed is not to be fed to swine weighing more than 75 pounds body weight. The finished feed is not to be fed to swine within 10 weeks of slaughter. The Type A medicated article is not to be mixed in complete feeds containing less than 15 percent crude protein.

IV. EFFECTIVENESS: No information was required for this supplemental approval.

V. TARGET ANIMAL SAFETY: No information was required.

VI. HUMAN FOOD SAFETY

A. TOXICITY STUDIES

The 36th Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) has evaluated the toxicology data for carbadox and its major metabolites. These data clearly show that the endpoint of toxicological concern for carbadox is carcinogenicity. Studies relating to the carcinogenic potential of carbadox and its metabolites are summarized.

Carbadox

Genotoxicity Studies

Carbadox was evaluated by the sponsor and others in standard genotoxicity batteries. Positive results were seen in the Ames test with *Salmonella typhimurium* TA1535, TA100, TA98 (+/-S9) and equivocal results were seen in the Ames test in *Salmonella typhimurium* TA1536, TA1537, TA1538 and C340. Mutagenicity Ames test was positive for *E. coli* WP2hcr, TA100 (+/- S9), and TA 98 and negative for TA1535, TA1537, and TA1538. The host-mediated assay was positive for *S. typhimurium* and negative for TA1534 and TA1952. The repair test (*Bacillus subtillis* [rec] and *S. typhimurium* [urv]) and the fluctuation test (*Klebsiella pneumoniae* and *E. coli*) were positive. The mutagenicity test (*Saccharomyces cerevisiae* D4), micronucleus test (rat bone marrow), repair test (*Bacillus subtillis* M45) and chromosomal damage tests (mouse bone marrow and *in vitro* human lymphocytes) were positive. The dominant lethal test in CD-1 mice was negative (Pfizer Central Research, undated; Negishi *et al.*, 1980; Ohta *et al.*, 1980; Voogd *et al.*, 1980; Beutin *et al.*, 1981; Yoshimura *et al.*, 1981; and Cihak and Srb, 1983).

Long-term/Carcinogenicity Studies

Rats

One hundred twenty Charles River C-D rats were divided into 6 groups (10/sex/dose) and received carbadox in the diet at rates providing 100, 50, 25, 10, 5, and 0 mg carbadox/kg b.w./day for 26 months. Hematology and urinalysis were evaluated in 5 rats/sex/dose at 3, 6, 12, 18 and 25 months. Rats were sacrificed at 14 and 112 weeks and received gross necropsies. Tissues were examined histopathologically (Stebbins, 1964).

At the interim sacrifice (14 weeks), rats in the 100 mg /kg b.w./day group showed decreased weight gain and food consumption, reduced hemoglobin, RBCs and neutropenia. Microscopic changes included pulmonary hemorrhage and edema, adrenal cortical hemorrhage and degeneration, splenic hemosiderin and thymic atrophy. Rats in the 50 mg /kg b.w./day group showed decreased weight gain and food consumption. Microscopic changes included pulmonary hemorrhage and edema, adrenal cortical hemorrhage, necrosis and degeneration, splenic hemosiderin and renocorticomedullary fatty metamorphosis. All of the rats in the 100 and 50 mg /kg b.w./day groups were sacrificed at 14 weeks. In the remaining treatment groups, 3

rats/sex/dose were sacrificed at 14 weeks. In the 25 mg /kg b.w./day group, rats had reduced weight gain, slight adrenal cortical atrophy, degeneration/necrosis and renal tubular fatty change. Rats in the 5 and 10 mg /kg b.w./day groups had no clinical, gross or microscopic changes reported at 3 months (Stebbins, 1964).

The remaining rats were evaluated from 3 months up to and including the 26-month sacrifice. In the 25 mg /kg b.w./day group, one female rat died at 51 weeks with no drug-related changes noted. All 13 remaining rats were sacrificed at 73 weeks due to palpable abdominal masses. At necropsy, all rats had multiple hepatic nodules. Ten of 13 rats were diagnosed microscopically as having benign nodular hyperplasia while the remaining 3 rats were determined to have malignant transformation bases on metastatic foci in other organs. In the 10 mg /kg b.w./day group, one rat died after 67 weeks with reticuloendothelial neoplasia, a common tumor of aged rats. The remaining rats in the group were sacrificed between the 64th and 112th week. Eleven of 13 rats were found to have hepatic benign nodular hyperplasia. In the 5 mg /kg b.w./day group, one male died at 20 weeks due to pulmonary abscesses. The remaining 13 rats died or were sacrificed between the 61st and 112th week. Five of the rats were found to have hepatic benign nodular hyperplasia. One control rat was sacrificed at 33 weeks with a forestomach papilloma and pulmonary atelectasis. A second male died at 93 weeks with myocarditis, nephrosclerosis and peribronchitis. The remaining rats were sacrificed between the 80th and 112th week. None of the control animals were observed to have hepatic benign nodular hyperplasia (Stebbins, 1964).

In a study to determine the level of carbadox tolerated by rats chronically, 120 Charles River rats received 2.5, 1.0 or 0 mg/kg b.w./day of carbadox in the diet. Hematology, urinalysis and ophthalmoscopic examinations were done at 3, 6, 12, 18 and 24 months on 5 rats/sex/dose. Interim sacrifices of 5 rats/sex/dose were conducted at 54 weeks with the remainder of the rats being sacrificed at 106 weeks. All evaluated gross necropsies and microscopic parameters were within normal limits at 54 weeks. At 2 years in the 2.5 mg /kg b.w./day group, 7/27 rats displayed hepatic benign nodular hyperplasia and peliosis hepatis. Additionally, the 2.5 mg /kg b.w./day group showed an increase in total mammary tumors. In the 1.0 mg /kg b.w./day group, 1/29 rats had hepatic benign nodular hyperplasia and 3/29 displayed peliosis hepatis. In the control group, 3/29 rats had hepatic benign nodular hyperplasia and 2/29 displayed peliosis hepatis. The 1.0 mg /kg b.w./day dose was tolerated by rats for 2 years with no adverse effects (Sigler, 1969).

In a third study, rats (14/sex/group) were treated with 25 mg/kg b.w./day carbadox or one of two lots of desoxycarbadox in the feed for 10 months to compare the oncogenic activity of carbadox and desoxycarbadox. An equal sized group served as untreated controls. Two to four rats were sacrificed at 30, 60, 90, 191 and 309 days. Animals received a gross necropsy at sacrifice and liver and adrenals were evaluated histopathologically. A moderate decrease in body weight occurred in both sexes receiving carbadox. At necropsy, all groups treated for 10 months showed evidence of hepatic changes including necrosis and nodule formation. Changes in the carbadox-treated group were less severe than the changes in the desoxycarbadox groups. All desoxycarbadox-treated rats and 2/18 of the carbadox-treated rats showed evidence of hepatocellular carcinoma. There was a significant increase in adrenal cortical hemorrhage in all treatment groups. In this study, carbadox induced tumors in rats (King, 1976).

Monkeys

The long-term toxicity of carbadox in primates was assessed. Twenty-eight monkeys were divided into 4 groups of 7 animals (3 or 4 *per* sex) and were dosed with carbadox orally in gelatin capsules. Doses were 20 mg/kg b.w./day (as 5 mg/kg b.w./day QID), 10 mg/kg b.w./day (as 5 mg/kg b.w./day BID) or 5 mg/kg b.w./day and controls. Animals were evaluated at 1, 3, 6, 12 and 24 months. Animals were sacrificed at 3 months and 2 years.

Elevated transaminase levels were detected at the 3- and 6-month evaluations. The monkeys tolerated 20 mg/kg b.w./day for 2 years with no adverse effects (Coleman, 1967).

Summary

Using data from these studies, a low-dose linear statistical model was used to determine an S_0 of 106 ppt for carbadox.

Desoxycarbadox

Genotoxicity Studies

Desoxycarbadox was evaluated by the sponsor in standard genotoxicity test batteries. Negative results were seen in the Ames tests with TA1535, TA1537, TA1538, TA100, TA98, TA1537+S9 and TA1535 +S9. The host-mediated assay was negative for TA1950, for TA1950 in mice and rats, and TA1535 in rats. The Ames test was positive for TA1535+/- TA100S9(rat) and for TA1535+S from rat and mouse. The Ames test was negative TA1535+S9 from hamster, dog and monkey. The chromosomal damage test was negative for human lymphocytes and rat bone marrow in a 5-day test. The chromosomal damage test was positive for rat bone marrow in a 9-month feeding test. The cell transformation test in BALB/C Swiss 3T3 was positive (Pfizer Central Research, 1975; Holmes, 1976).

Long-term/Carcinogenicity Studies

Rats

A long-term study was conducted by the sponsor to determine the tumorigenic potential of desoxycarbadox, a carbadox metabolite. Four hundred Charles River C-D rats were divided into groups of 50/sex/dose. Desoxycarbadox was administered continuously in the diet at doses of 0, 5, 10 and 25 mg/kg b.w./day. Although treatment originally was scheduled for 2 years, test material was withdrawn from all rats in the 25 mg/kg b.w./day and 50 percent of the rats of each sex in the other two treatment groups on Day 350 due to high morbidity and mortality. Administration of desoxycarbadox was stopped completely on Day 416. The study was terminated on Day 447 (Reinert, 1976).

Clinical examinations revealed a number of treatment-related signs including tumors in the mammary region in both sexes, small cutaneous nodules, and enlargement of the liver with nodules preceded by weight loss and polyphagia. There was a dose-related decrease in weight gain and a dose-related decrease in survival. Clinical chemistry parameters were evaluated. Desoxycarbadox resulted in increases in plasma enzyme

activity, urea and bilirubin, abnormalities consistent with hepatic disorders. The results were highly variable and nonreversible following drug withdrawal. A dose related hypoglycemia was noted in both sexes. Hematological parameters were evaluated in 10 rats/sex/group at Day 413 and for the remainder on Day 447. A moderate hypochromic microcytic anemia was seen in the 25 mg/kg b.w./day groups and a slight anemia was seen in the 10 mg/kg b.w./day. A neutrophilia also was observed in the 25 mg/kg b.w./day (Reinert, 1976).

All rats received a gross necropsy. Histopathological examinations were performed on all grossly abnormal tissue and routinely on a standard array of tissues. There was a dose-related increase in pigmentation of the renal tubules and nephrosis. An increased tumor incidence was seen in all treatment groups. Desoxycarbadox is a potent hepatocarcinogen and there were dose-related increases in other tumors (Reinert, 1976).

Summary

Using data from these studies, a low-dose linear statistical model was used to determine an S_0 of 61 ppt for desoxycarbadox.

Hydrazine

Genotoxicity Studies

Hydrazine was evaluated by several researchers in standard genotoxicity batteries. Positive results were seen in the *Salmonella typhimurium* Ames test, the mouse lymphoma cell test and the bacterial test using *E. coli* WP2 uvr A trp (Ames, 1971; von Wright and Tikkanen, 1980; and Rogers and Back, 1981).

Long-term/Carcinogenicity Studies

Mice

A number of long-term/carcinogenicity studies have been conducted in mice to evaluate the toxicity of hydrazine. These published studies are briefly summarized for completeness.

Oral administration of hydrazine to BALB/C female mice at 1.13 mg/day for 46 weeks produced a 100 percent incidence of lung tumors (Biancifiori and Ribacchi, 1962).

A 46 percent incidence of lung tumors was seen in female Swiss mice treated with hydrazine at a dose level of 0.25 mg/day for 5 days/week for 46 weeks. Control mice had a 10 percent incidence (Roe *et al.*, 1967).

In a study with CBA/Cb/Se mice, hydrazine was administered by gavage at a dose of 1.13 mg/day for 36 weeks. Treated mice had an incidence of 76 percent and 90 percent lung tumors for males and females, respectively. Control mice had an incidence of 3 percent. Hepatomas were found in 62 percent of males and 71 percent of the females treated with hydrazine. Control mice had an incidence of 11 percent and 4 percent for males and females, respectively (Severi and Biancifiori, 1968).

CBA mice were divided into groups of 40-59 mice /sex. Hydrazine was administered by gavage daily for 150 days at rates of 45, 22, 11, 5.6 and 0 mg/kg b.w./day. Mice were examined at natural death or following sacrifice when moribund. Control males had a hepatoma incidence of 10 percent while females had a 3.4 percent rate. Treated males had hepatoma rates of 60, 48, 28 and 3.8 percent, at 45, 22, 11, 5.6 mg/kg b.w./day, respectively. Treated females had hepatoma rates of 62.5, 66.6, 8 and 0 percent, respectively (Biancifiori, 1970).

Hamsters

Golden hamsters were divided into three groups of 23, 35 and 56 animals. Animals received 60 doses of 3.0 mg hydrazine *via* intubation over a 15-week period, 100 doses of 2.8 mg hydrazine *via* intubation over a 20-week period, or no hydrazine, respectively. Hepatic lesions, including fibrosis, reticuloendothelial cell proliferation and bile duct proliferation, were found in 60-80 percent of the treated hamsters but in none of the control animals (Biancifiori, 1970).

Negative tumorigenicity results were obtained in a chronic study in golden hamsters. Fifty hamsters/sex received hydrazine in the drinking water at 2.3 mg/day for a lifetime (Toth, 1972).

Rats

A chronic oral study was conducted in Cb/Se rats. Hydrazine was administered daily by stomach tube at doses of 18 mg/rat to 14 males and 12 mg/rat to 18 females. Dosing continued for 68 weeks. Lung tumors were found in 21 and 27 percent of the dosed male and female rats, respectively. Control groups (28M, 22F) had no lung tumors. Hepatic tumors were found in 30 percent of the treated males while no hepatic tumors were found in treated females or controls (Severi and Biancifiori, 1968).

Summary

Based on these published data, FDA has concluded that hydrazine induces tumors in animals. Using a low-dose linear statistical model, an S_0 of 11 ppb is calculated.

Methyl Carbazate

Genotoxicity Studies

Methyl carbazate was evaluated in standard genotoxicity batteries. Negative results were seen in the Ames tests and the chromosomal damage test. Results in the host-mediated assay were equivocal (Pfizer Central Research, 1975; Holmes, 1976).

Long-term/Carcinogenicity studies

Rats

The chronic oral toxicity of methyl carbazate, a metabolite of carbadox, was studied by the sponsor in the rat. Wistar rats were divided into groups of 12/sex/dose and received methyl carbazate at target doses of 1 and 10 mg/kg b.w./day in feed for 10 months. Necropsies were performed on all animals and histology was conducted on a standard

array of tissues and all tissues with grossly observed tumors. Three males and three females in the high dose group died before termination. No histopathological evidence of toxicity or evidence of elevation in tumors was reported. The administration of methyl carbazate to rats at dose levels of 1 and 10 mg/kg b.w./day produced no evidence of carcinogenic potential (Rutty, 1972).

In a second study conducted by the sponsor, Wistar rats were divided into groups of 24/sex and were treated with target doses of 0, 2.5, 5 and 10 mg/kg b.w./day in the feed for 710 days. Clinical examinations were conducted weekly. Blood samples for clinical chemistry were obtained at terminal sacrifice and from moribund animals. Hematology was conducted on samples from moribund and dead rats as well as from an interim sacrifice of 6 rats at 12 months. Rats were sacrificed and necropsied at 710 days. Histopathology was performed on all gross lesions and on a standard array of tissues. There were no treatment related effects noted in any parameters evaluated in the study. There was histological evidence of widespread chronic respiratory disease in all groups. Methyl carbazate had no adverse effect when given to rats in the diet for 2 years (Ferrando, 1980).

Summary

On the basis of these studies, the agency has concluded that methyl carbazate does not induce tumors in animals.

Quinoxaline-2-Carboxylic Acid (QCA)

Genotoxicity Studies

Quinoxaline-2-carboxylic acid was evaluated by the sponsor in a standard genotoxicity battery. Negative results were seen in the Ames tests with *Salmonella typhimurium,* TA1535, TA1537, TA1538 and TA1535+S9 tests. The chromosomal aberration test in *in vitro* human lymphocytes also was negative (Pfizer Central Research, 1975).

Long-term/Carcinogenicity Studies

Mouse

Charles River CD mice (50/sex/dose) received QCA in feed for 19 months at levels to deliver 0, 25, 50 and 100 mg/kg b.w./day. Hematology and clinical chemistry were evaluated once, prior to sacrifice. Necropsies were performed on all animals and histopathological examinations were conducted on a standard array of tissues. No treatment-related effects were noted in any parameter during the study. Cumulative mortalities and incidences of tumors were comparable for the control and treatment groups. Oral administration of QCA to mice for 19 months produced no evidence of toxicity (Faccini *et al.*, 1979).

Rats

Nine male and 9 female Charles River C-D rats were divided into groups of 3 rats/sex/dose. Rats received QCA in the feed for 2 years at levels to provide 100, 50 or 0 mg/kg b.w./day. Rats received clinical examinations weekly and routine ophthalmoscopic, hematology and urinalysis examinations. Terminal sacrifice was

performed on Day 735 of the study. All rats received gross necropsies and standard tissues were evaluated microscopically. No treatment-related changes were reported and QCA was tolerated at up to 100 mg/kg b.w./day when given to rats *via* feed (Coleman, 1971).

In a study to determine whether QCA has tumorigenic potential, Charles River Sprague-Dawley rats (20/sex/dose) were treated with QCA in the diet for two years at doses to provide 0, 10, 25, and 50 mg/kg b.w./day. Rats received clinical examinations weekly and routine ophthalmoscopic, hematology and urinalysis examinations. At 12 months, 5 rats/sex/dose and at 24 months all remaining rats were sacrificed, received a gross necropsy and standard tissues were examined histopathologically. No treatment-related effects were noted for any of the evaluated parameters. Cumulative tumor rates were comparable for control and treated rats. QCA in doses of 10, 25, and 50 mg/kg b.w./day over a 2- year period does not produce any toxicity or elevated tumor incidence (Pfizer Central Research, 1971).

Summary

Thus, quinoxaline-2-carboxylic acid is not a carcinogen in animals.

B. DEFINITION OF "NO RESIDUE"

Neither an ADI nor a safe concentration of total residues is calculated for carbadox. Carbadox, and its metabolites desoxycarbadox and hydrazine, were determined to be carcinogenic in animals. Methyl carbazate and quinoxaline-2-carboxylic acid were also tested in short-term genotoxicity assays and carcinogenicity studies and these compounds are not carcinogens.

Pursuant to 21 CFR 500.84(c)(2), FDA considers that "no residue" of a compound remains in edible tissue when the residue of carcinogenic concern in the total diet of people does not exceed S_0. The S_o is defined in 21 CFR 500.82(b) and 500.84 (c)(1) as the concentration of total residue of carcinogenic concern of the test compound in the total diet of test animals that corresponds to a maximum lifetime risk of cancer in the test animals of 1 in 1 million. For carbadox and each of the carcinogenic metabolites, an S_o was calculated using a low-dose linear extrapolation statistical model. A S_o of 106 parts per trillion, 61 parts per trillion, and 11 parts per billion was calculated for carbadox, desoxycarbadox, and hydrazine, respectively. Based on these results, a S_o of 61 parts per trillion (ppt) is determined for the total residues of carcinogenic concern for carbadox in the total diet. As provided in 21 CFR 500.82(b), this concentration, *i.e.*, the S_0, represents no significant increase in risk of cancer to people.

Because the total human diet is not derived from food producing animals, a correction for food intake is made in determining the concentration of residues of carcinogenic concern that will be permitted in edible animal tissue, 21 CFR 500.84(c)(2). FDA designates as S_m the permitted concentration of residues of carcinogenic concern in a specific edible product, 21 CFR 500.82(b) and 500.84 (c)(2). Given a 1500 g total daily diet in humans, it is assumed that up to 500 g is due to the consumption of meat. Of this 500 g total daily meat consumption, 300 g is assumed to be comprised of muscle, 100 g comprised of liver, 50 g comprised of kidney, and 50 g comprised of fat. Thus, for any calculated S_0, one fifth of the total diet (or 300*g muscle*/1500*g total diet*) would be comprised of muscle. For an S_0 of 61 parts per trillion (ppt), the concentration of

total residues of carcinogenic concern, $S_{m\text{-}muscle}$, would then be calculated as 61 ppt ÷ 1/5 or 305 ppt. The S_m for each edible tissue is calculated as follows:

Table 1. Consumption values and calculated S_m for residues of carcinogenic concern in edible swine tissues

Tissue	Consumption Factor	Fraction of Total Diet	S_0	S_m
muscle	300 g	1/5	61 ppt	305 ppt*
liver	100 g	1/15	61 ppt	915 ppt
kidney	50 g	1/30	61 ppt	1830 ppt or 1.83 ppb**
fat	50 g	1/30	61 ppt	1830 ppt or 1.83 ppb**

*parts per trillion

**parts per billion

C. Residue and Metabolism Studies

Introduction

The sponsor and academic researchers have conducted numerous studies evaluating the fate of carbadox in animals. These residue depletion data are summarized in FAO Food and Nutrition Paper 41/3 (Food and Agriculture Organization (FAO) of the United Nations, 1991) and show that carbadox, desoxycarbadox and hydrazine do not persist in edible tissue as detectable residues beyond 72 hours. The agency's evaluation of these data, and the new information provided by the sponsor, demonstrate that following administration, parent carbadox is rapidly metabolized; that the metabolism of carbadox is similar among species; that the *in vivo* metabolism of the compounds of carcinogenic concern is also rapid and irreversible such that the resulting metabolic products cannot regenerate compounds of carcinogenic concern; that the unextractable residues are related to non-carcinogenic compounds, quinoxaline-2-carboxylic acid and quinoxaline-2-carboxaldehyde; and that quinoxaline-2-carboxylic acid is the only residue detectable in the edible tissues beyond 72 hours post dosing. Thus, the agency concludes that the unextractable bound residue is not of carcinogenic concern and that QCA is a reliable marker residue for carbadox.

The sponsor has conducted two total residue and metabolism studies in swine to establish the marker residue for carbadox in swine liver. In the first radiotracer study, the swine developed an enteritis on Day 2 of the treatment period that persisted for 2 days post-treatment. Feed consumption was reduced in the sick animals. The second study was conducted to address deficiencies in the first study resulting from the presence of enteritis in the test animals.

Total Residues in Swine

1. A ^{14}C-Carbadox Radiotracer Tissue Residue Study in Growing Swine

The final report is No. 1525N-60-87-004, conducted by MJ Lynch, Pfizer Central Research. The report is dated February 1988.

Ten preconditioned crossbred swine (5M and 5F) weighing 30 kg were used in the study. The pigs were identified by ear tags and were given *ad libitum* access to medicated feed containing 55 ppm ring-labeled ^{14}C-carbadox for five consecutive days. The radiolabeled drug used to prepare the medicated feed had a specific activity of 8.4 µCi/mg and a radiopurity (HPLC and TLC) of more than 99 percent. Following the treatment period, the swine were maintained on a basal ration pending sacrifice at 30, 45 or 70 days withdrawal. Two untreated swine served as controls. Excreta were collected during the treatment period and for 2 days following return to the basal ration. At necropsy, samples of the four edible tissues (500 g each) were collected. Following homogenization, the tissue samples were assayed for total and bound radioactivity and for major metabolites. Total radioactivity was determined *via* combustion and liquid scintillation counting. Bound residues were assessed following organic extraction, with and without acid. Quinoxaline-2-carboxylic acid (QCA), the only metabolite present at the sampling times, was determined by thin-layer, gas chromatography reverse isotope dilution analysis, following derivatization to methyl quinoxaline-2-carboxylate.

Table 2. Total radioactivity (ppm ^{14}C-carbadox equivalents) in tissues of swine following five days of feeding ^{14}C-carbadox at 55 ppm

Days Postdosing	Liver	Kidney	Muscle	Fat
30	44.7±27.0	14.5±4.9	6.7±2.5	<2
45	12.3±3.8	4.0±2.0	1.7±0.6	<2
70	4.0±1.6	1.8±0.5	<1	<2

Table 3. Percentages of radioactivity remaining in tissue following organic extraction without acid

Days Postdosing	Liver	Kidney	Muscle	Fat
30	93.8±1.1	93.0±1.3	95.4±3.2	N/A*
45	94.2±1.8	94.6±4.7	92.1±3.2	N/A

*N/A = Not assayed

Table 4. Percentages of radioactivity extracted from tissue following digestion with 1M HCl

Days Postdosing	Liver	Kidney	Muscle	Fat
30	2.3±0.9	1.8±0.3	2.3±0.7	N/A*
45	3.0±0.2	7.2±5.5	3.5±3.5	N/A

*N/A = Not assayed

2. A ^{14}C-Carbadox Radiotracer Tissue Residue Study in Growing Swine

The final report is No. 1525N-60-87-005, conducted by MJ Lynch, Pfizer Central Research. The report is dated February 1988.

Ten preconditioned crossbred swine (5M and 5F) weighing 30 kg were used in the study. The pigs were identified by ear notch and were given *ad libitum* access to medicated feed containing 55 ppm ring-labeled ^{14}C-carbadox for five consecutive days. The radiolabeled drug used to prepare the medicated feed had a specific activity of 8.4 µCi/mg and a radiopurity (HPLC and TLC) of more than 99 percent. Following the treatment period, the swine were maintained on a basal ration pending sacrifice at 30, 45 or70 days withdrawal. One untreated female pig served as the control. Excreta were collected during the treatment period and for 2 days following return to the basal ration. At necropsy, samples of the four edible tissues (500 g each) were collected. Following homogenization, the tissue samples were assayed for total and bound radioactivity and for major metabolites. Total radioactivity was determined *via* combustion and liquid scintillation counting. Quinoxaline-2-carboxylic acid (QCA), the only metabolite present at the sampling times, was determined by thin-layer, gas chromatography reverse isotope dilution analysis, following derivatization to methyl quinoxaline-2-carboxylate.

Table 5. Total radioactivity (ppb[1] ^{14}C-carbadox equivalents) in tissues of swine following five days of feeding ^{14}C-carbadox at 55 ppm

Days Postdosing	Liver	Kidney	Muscle	Fat
30	74.5±30.5	15.3±5.1	5.0±1.4	2.3±1.0
45	20.0±2.8	5.0±1.0	3.0±1.0	<1
70	13.3±0.6	3.7±0.6	2.3±0.6	<1

[1] 1. The revised units are (1) different from the units (ppm) that were published in the original FOI Summary, and (2) the sole revision in the document.

Metabolic Profiling in Swine

The profiling of carbadox metabolites in swine was conducted with tissues from a preliminary total residue study.

In swine treated once with ^{14}C-carbadox following a stress period with cold drug, peak radioactivity in plasma was observed at 3 hours post dosing. In plasma collected five hours post treatment, carbadox (13 percent of total), desoxycarbadox (19 percent of total), aldehyde (13 percent of total) and QCA (19 percent of total) were present. At 8 hours post treatment, only desoxycarbadox (9 percent of total) was identified in swine plasma. All four compounds had disappeared within 24 hours.

About two-thirds of the dose was eliminated in the urine, the remainder in the feces. For the 0-24-hour post-dosing interval, urine radioactivity averaged 65.4 percent and fecal radioactivity averaged 7.6 percent for a total average 0-24-hour excretion of 73.1 percent. For the 24-48-hour postdosing interval, urine radioactivity averaged 2.4 percent and fecal radioactivity averaged 10.2 percent for a total average 24-48-hour excretion of 12.6 percent and a 0-48-hour excretion of 85.4 percent. A small percentage of radioactivity was excreted over the 48-72hour period (0.4 percent, urine, and 1.1 percent feces) for a total 0-72 excretion of 88.2 percent. Urinary metabolites of carbadox were assayed by TLC and radiography. Quinoxaline-2-carboxylic acid was identified as the major metabolite in swine urine. It was present in a free form and as its glycine conjugate. No N-oxides were found in urine. In feces, 9 percent of the radioactivity was attributable to QCA and no unchanged carbadox was detected.

When studies were conducted with carbonyl-labeled carbadox, methyl carbazate is generated. Approximately 25 percent is excreted in the urine. Most of the methyl carbazate is enzymatically cleaved to yield CO_2 which is exhaled. Radioactivity in the liver decreased with a half-life of two days. At 5 days post dosing, liver radioactivity corresponded to 0.12 ppm methyl carbazate equivalents, consisting in part of amino acids labeled by incorporation of $^{14}CO_2$. Enzymatic hydrolysis of methyl carbazate implies but does not prove the formation of hydrazine. No radiotracer method can demonstrate the absence of hydrazine-related residues (*i.e.*, hydrazine = $H_2N\text{-}NH_2$). Chemical assays strongly suggest, however, that hydrazine does not form a significant tissue residue. In plasma, hydrazine is not detected by an assay sensitive to 0.1 ppm. This is not unexpected since several enzymatic processes are known to destroy hydrazine.

Residues of QCA were determined in the tissues collected from studies Nº. 1525N6087-004 and Nº. 1525N-60-87-005.

Table 6. Residues (ppb) of methyl quinoxaline-2-carboxylate (expressed as ppb ^{14}C-carbadox equivalents) in the tissues of swine (No. 1525N-60-87-004)

Days Postdosing	Liver	Kidney	Muscle	Fat
30	9.3±6.7	<1	<1	N/A*
45	2.7±0.7	<1	<1	
70	0.3±0.1	<1	<1	N/A

*N/A = Not assayed

Table 7. Residues (ppb) of methyl quinoxaline-2-carboxylate (expressed as ppb ^{14}C-carbadox equivalents) in the tissues of swine (Nº. 1525N-60-87-005)

Days Postdosing	Liver	Kidney	Muscle	Fat
30	18.9±10.4	N/A	N/A	N/A*
45	5.5±1.1	N/A	N/A	N/A
70	1.3±0.5	N/A	N/A	N/A

*N/A = Not assayed

D. Comparative Metabolism Studies

The metabolism of carbadox was studied in monkeys and rats. The animals were treated orally with ^{14}C-carbadox having a specific activity of 1.97 µCi/mg. Rats were given 5 mg/kg by stomach tube and one monkey was given 5 mg/kg in a capsule. Urine and feces were collected and assayed for total radioactivity. Urinary metabolites of carbadox were compared qualitatively by TLC and radiography.

The majority of the metabolites found in the pig also were found in the rat or the monkey. One metabolite not found in the monkey or the rat is the glycine conjugate of QCA; the other metabolite represented only a few percent of the total urinary radioactivity.

Table 8. Excretion pattern (0-72 hrs) of radioactivity (expressed as percent of dose) found in swine, monkey and rats, after ingestion of ^{14}C-carbadox.

Species	Dose	Urine	Feces
Swine	3.5 mg/kg	74.1	16.5
Monkey	5 mg/kg	61.3	7.5
Rats (6)	5 mg/kg	54.0	N/A*

E. Designation of a Marker Residue and Tolerance

According to metabolic studies on carbadox, desoxycarbadox, and hydrazine, which demonstrate that these compounds do not persist in swine muscle, liver or kidney beyond 72 hours, the assignment of quinoxaline-2-carboxylic acid (QCA) can serve as the marker residue for the residues of carcinogenic concern. The assignment of a tolerance of 30 ppb for QCA in swine liver assures that all residues of carcinogenic concern are well below their respective S_0 in all edible tissues. Therefore, no residues of carcinogenic concern remain in the carcass when using an assigned value of 30 ppb for the noncarcinogenic marker residue (QCA).

The average percentage of total residues represented by quinoxaline-2-carboxylic acid was determined from the two radiolabeled metabolism studies in swine (see Section C above).

Table 9. The average percent of total residues represented by QCA

Days Post Dosing	Total Residue	QCA	Percent QCA
30	75 ppb	18.9ppb	24.4
45	20 ppb	5.5 ppb	27.5
70	13 ppb	1.3 ppb	9.9

F. Regulatory Method

Residues of quinoxaline-2-carboxylic acid are determined using a gas chromatographic assay with electron capture detection. The method has a limit of quantification of 5 ppb. The method is on file at the Center for Veterinary Medicine, Food and Drug Administration, HFV-199, 7500 Standish Place, Rockville, Maryland 20855.

VII. AGENCY CONCLUSIONS

The Federal Food, Drug, and Cosmetic Act requires that sponsored compounds intended for use in food-producing animals be shown to be safe and that food produced from animals exposed to these compounds be shown to be safe for consumption by people. The statute prohibits the use in food-producing animals of any compound found to induce cancer when ingested by people or animals unless it can be determined by methods of examination prescribed or approved by the Secretary by regulation (a function delegated to the Commissioner of Food and Drugs under §5.10 of this chapter) that no residue of that compound will be found in the food produced from those animals under conditions of use reasonably certain to be followed in practice. The regulations describing the operational definition of "no residue" are found in 21 CFR 500.84.

On the basis of the results of the chronic bioassays and other information submitted by the sponsor, FDA determined that carbadox and its metabolites, desoxycarbadox and hydrazine, that are formed on food as a result of the use of carbadox, are carcinogenic. For each of these substances, FDA has decided they must be regulated as carcinogens. Each carcinogenic substance has been tested in separate bioassays. FDA has analyzed the data from the bioassays using a statistical extrapolation procedure to calculate the concentration of the residue of carcinogenic concern that corresponds to a maximum lifetime risk to the test animal of 1 in 1 million. FDA designates the lowest value obtained as the S_0, that is, the S_0 of 61 ppt for desoxycarbadox.

FDA considers that "no residue" of the compound remains in the edible tissue when conditions of use of the sponsored compound, including any required preslaughter withdrawal period (currently 70 days), ensure that the concentration of the residue of carcinogenic concern in the total diet of people will not exceed S_0. Because the total diet is not derived from food producing animals, FDA has made corrections for food intake. FDA has designated S_m the concentration of residue of carcinogenic concern that is permitted in a specific edible product. The S_m for total carbadox residues of carcinogenic concern are derived from the S_0 of 61 ppt and equal 305 ppt in muscle, 915 ppt in liver, 1.83 ppb in kidney and fat.

For each edible tissue, the sponsor measured the depletion of the residue of carcinogenic concern until its concentration is at or below S_m. The sponsor also measured the depletion of the potential marker residues until the concentration of the residue of carcinogenic concern is at or below S_m in all the edible tissues.

Under FDA's operational definition of "no residue," a residue of carcinogenic concern, so long as it does not exceed the S_0, may be detectable by an approved method. The residue data show that carbadox, desoxycarbadox and hydrazine do not persist in edible tissue as detectable residues beyond 72 hours. The *in vivo* metabolism of the compounds of carcinogenic concern is irreversible. Therefore, in this case, no residue of carcinogenic concern, even below the S_0, is detectable by any method. The unextracted residues are related to a noncarcinogenic compound, quinoxaline-2-carboxylic acid (QCA), and extractable QCA is the only residue detectable in the edible tissues 72 hours postdosing. Thus, the agency concludes that QCA is a reliable marker residue for carbadox and its metabolites.

From these data, FDA has selected liver as the target tissue and quinoxaline-2-carboxylic acid (QCA) as the marker residue. FDA has determined that when QCA, the marker, is at

or below 30 ppb in the target tissue, liver, that no residue of carcinogenic concern, above the S_0, is detectable in each of the edible tissues by any method.

The sponsor has submitted a regulatory method capable of measuring QCA at and below 30 ppb in the target tissue.

REFERENCES

Ames, B. (1971). Chemical Mutagens, Vol. 1, Hollaender, A. (ed), Plenum Press, New York. p 267.

Beutin, L., Preller, E., and Kowalski, B. (1981). Mutagenicity of quindoxin, its metabolites, and two substituted quinoxaline-di-N-oxides. *Antibacterial Agents and Chemotherapy,* **20**, 336343.

Biancifiori, C., and Ribacchi, R. (1962). Pulmonary tumour in mice induced by oral isoniazid and its metabolites. *Nature*, **194**, 488-489.

Biancifiori, C. (1970). Hepatomas in CBA/Cb/Se mice and liver lesions in golden hamsters induced by hydrazine sulfate. *J. Nat. Cancer Inst.*, **44**, 943-953.

Cihak, R., and Srb, V. (1983). Cytogenic effects of quinoxaline-1,4-dioxide-type growthpromoting agents. *Mutat. Res.* **116**, 129-135.

Coleman, G.L. (1967). Two year chronic study of GS-6244 (carbadox) in monkeys. Unpublished. Submitted to WHO by Pfizer Central Research, Groton, CT, USA.

Coleman, G.L. (1971). Quinoxaline-2-carboxylic acid, first two year study in rats. Unpublished. Submitted by Pfizer Central Research, Groton, CT, USA.

Faccini, J.M., Perraud, J., Nachbaur, J., and Monro, A.M. (1979). 19-Month toxicity study in mice. Unpublished. Submitted by Pfizer Central Research, Groton, CT, USA.

Ferrando, R. (1980) Two year chronic toxicity study in the rat with methyl carbazate. Unpublished. Submitted by Pfizer Central Research, Groton, CT, USA.

Food and Agriculture Organization (FAO) of the United Nations (1991). Residues of some veterinary drugs in animals and foods, 41/3, Rome, February 5-14, 1990.

Holmes, C.L. (1976). Addendum genetic toxicology report CP-17,056 (desoxycarbadox). Unpublished. Submitted by Pfizer Central Research, Groton, CT, USA.

King, T.O. (1976). Target organ toxicity of GS-6244 (carbadox) and CP-17,056 (desoxycarbadox) with chronic administration in rats. Unpublished. Submitted by Pfizer Central Research, Groton, CT, USA.

Negishi, T., Tanaka, K., and Hyatsu, H. (1980). Mutagenicity of carbadox and several quinoxaline-1,4-dioxide derivatives. *Chem. Pharm. Bull.* **28**, 126-128.

Ohta, T., Moriya, M., Kaneda, K., Watanabe, T., Miyazawa, A., and Susigyma, F. (1980). Mutagenicity screening of feed additives in the microbial system. *Mutat. Res.* **77**, 21-30.

Pfizer (1975). Genetic toxicology report CP-17,056 (desoxycarbadox). Unpublished. Submitted by Pfizer Central Research, Groton, CT, USA.

Reinert, H. (1976). Desoxycarbadox: Two year toxicity study in rats. Unpublished. Submitted by Pfizer Central Research, Groton, CT, USA.

Roe, F.J., Grant, G.A., and Milligan, D.M. (1967). Carcinogenicity of hydrazine and 1,1dimethylhydrazine in mouse lung. *Nature*, **216**, 375-376.

Rogers, A.M., and Back, K.C. (1981). Comparative mutagenicity of hydrazine and 3 methylated derivatives in L5178Y mouse lymphoma cells. *Mutat. Res.* **89**, 321-328.

Rutty, D.A. (1972). Twenty-one-month chronic toxicity study of methyl carbazate in rats. Unpublished. Submitted by Pfizer Central Research, Groton, CT, USA.

Severi, L., and Biancifiori, C. (1968). Hepatic carcinogenesis in CBA/Cb/Se mice and Cb/Se rats by isonicotinic acid hydrazine and hydrazine sulfate. *J. Nat. Cancer Inst.* **41**, 332-349.

Sigler, F.W. (1969). A two-year chronic toxicity study of GS-6244 in rats. Dose levels: 2.5, 1.0 or 0 mg/kg/day. Unpublished. Submitted by Pfizer Central Research, Groton, CT, USA.

Stebbins, R.B. (1964). A 3-week subacute toxicity study of GS-6244 in rats - Dose levels of 100, 50, 10, 5, or 0 mg/kg. Unpublished. Submitted by Pfizer Central Research, Groton, CT, USA.

Toth, B. (1972). Tumorigenesis studies with 1,2-dimethylhydrazine dihydrochloride, hydrazine sulfate and isonicotinic acid in golden hamsters. *Cancer Res.* **32**, 804-807.

Voogd, C.E., Van der Stel, J.J., and Jacobs, J.J. (1980). The mutagenic action of quindoxin, carbadox, olaquindox and some other N-oxides on bacteria and yeast. *Mutat. Res.* **78**, 233-242.

Von Wright, A., and Tikkanen, L. (1980). The comparative mutagenicities of hydrazine and its mono- and di-methyl derivatives in bacterial test systems. *Mutat. Res.* **90**, 49-53.

Yoshimura, H., Nakamura, M., Koeda, T., and Yoshikawa, K. (1981). Mutagenicities of carbadox and olaquindox - growth promoters in pigs. *Mutat. Res.* **90**, 49-55.