Journal of the American Association for Laboratory Animal Science
Copyright 2007
by the American Association for Laboratory Animal Science

Vol 46, No 6
November 2007
Pages 8–15

Overview

Biologic Effects of Fenbendazole in Rats and Mice: A Review

David Villar, Carolyn Cray,* Julia Zaias, and Norman H Altman

This review summarizes findings from toxicologic, carcinogenic, immunologic, and metabolic studies on fenbendazole (FBZ). Currently, FBZ is used to treat or prevent pinworm outbreaks in laboratory rodents. Because antiparasitic treatments usually are not part of experimental designs, interactions from the medication on the outcomes of ongoing experiments are a concern. At therapeutic levels, FBZ does not alter the total content of cytochromes P450 but does induce certain hepatic cytochrome P450 isoforms, namely 1A1, 1A2, and 2B1. Although expressed constitutively at low or undetectable levels, these isoforms particularly are known for bioactivating a number of procarcinogens. Lifetime studies in rats have shown that FBZ is not a carcinogen but that it may behave as a tumor promoter when given after certain initiators. Unlike in other animal species, FBZ treatment-associated myelosuppression has not been reported to occur in rodents. The few currently available immunologic studies in mice, including an autoimmune model, have not shown effects on selected immune responses. However, data from other animal species suggest that the ability of B and T lymphocytes to proliferate in the secondary immune response may be suppressed during treatment with FBZ.

Abbreviations: EROD, ethoxyresorufin O-deethylation; FBZ, fenbendazole; GST-P, glutathione S-transferase placental form; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline; MROD, methoxyresorufin O-deethylation; MTD, maximal tolerated dose; OXF, oxfenbendazole; ppm, parts per million

Fenbendazole (FBZ) is a broad-spectrum benzimidazole anthelminth currently approved for use in numerous animal species, including rodents. Although nematodes, and in particular pinworms (for example, *Syphacia* and *Aspicularis spp.*), are the main endoparasites of concern in laboratory rodents, FBZ also is indicated for use in other animal species against a wide spectrum of nematodes, tapeworms, flukes, and protozoa.[9,52]

Pinworm infestation in rodent laboratories occurs occasionally, and national surveys among large research institutions indicate these incidents are more prevalent than are infections by other organisms.[31] Although the parasites are relatively nonpathogenic, their presence may significantly alter the outcome of some laboratory experiments. For example, myelopoiesis and erythropoiesis are known to be increased in pinworm-infected mice and so may be the sensitivities of bone marrow progenitors to interleukins.[7] In addition, the stimulation of various immune responses, including autoimmune responses and elevated production of numerous interleukins, has been linked to infestation with pinworms.[1,42] In addition to the multiple effects on the immune system caused by parasitism, alterations in animal physiology that would be expected from conditions of mild chronic stress may occur, including changes on neuroendocrine responses, exploratory behavior, and growth of young animals.[41,68,75] Heavy infestations may cause overt clinical disease with signs of rectal prolapse, rough hair coats, and general poor body condition.[71] These studies highlight the importance of maintaining laboratory rodents under pinworm-free conditions.

Received: 20 Jun 2007. Revision requested: 4 Aug 2007. Accepted: 10 Sep 2007.
Division of Comparative Pathology, Miller School of Medicine, University of Miami, Miami, FL.
**Corresponding author. Email: ccray@med.miami.edu*

A review of the treatments available for the eradication of pinworm infestations from laboratory rodent colonies was published recently.[53] Of the various agents available, those currently used most frequently are avermectins (for example, ivermectin) and benzimidazoles (for example, FBZ).[53] Both drugs are 100% effective by the oral route and typically are delivered in the diet or drinking water. Ivermectin is very safe in adult animals, but it can cause toxicosis in young animals due to immaturity of the blood–brain barrier or in transgenic mice with P-glycoprotein deficiency.[30,53]

With regard to the benzimidazole group, FBZ-medicated feed has gained widespread use in pinworm prophylaxis and treatment protocols because of its large margin of safety and efficacy. From a practical standpoint, replacing regular feed with FBZ-medicated feed adds minimal personnel labor costs and can be implemented immediately. In addition, FBZ has adulticidal, larvicidal, and ovicidal actions.[53] Therapeutic levels can be administered for life without side or toxic effects, as summarized in a report by the World Health Organization.[77] However, as with any other drug, the question arises regarding whether FBZ causes physiologic changes that interfere with the outcomes of laboratory experiments. The following sections summarize biologic effects of FBZ in rodents that may be relevant to experimental protocols in biomedical research.

Use of FBZ in Rodents and General Safety Data

The recommended FBZ therapeutic dosages are usually lower for livestock species (5 to 10 mg/kg orally once daily for 3 to 5 d) than for pet animals, including birds and reptiles (20 to 100 mg/kg orally once daily for 3 to 10 d).[52] Commercially available med-

icated rodent diets contain FBZ at 150 parts per million to reach a target dosage of 8 to 12 mg/kg daily. In experiments where actual intake of medicated feed intake by rats was estimated, the mean daily consumption rate for male rats was 8.4 mg/kg and that for female rats was 11.5 mg/kg.[11] These feed concentrations, given in alternating weeks, effectively eradicated pinworms in rat and mouse colonies.[4,11,23,27,28] Although colony infestations have been eliminated without environmental decontamination or changes in husbandry practices, re-emergence of pinworms have occurred when apparently adequate surveillance programs and preventive measures failed.[4,27,28]

A World Health Organization Joint Expert Committee on Food Additives compiled a comprehensive report on FBZ that included an evaluation of unpublished acute toxicity studies and long-term toxicologic data in rodents.[19-21,60,61,77] FBZ can be considered a nontoxic drug because in rodents, the dose lethal to 50% of the tested population exceeds 10 g/kg (a dose 1000 times the therapeutic level). In a short-term (14 d) toxicity study using Sprague–Dawley rats (weight, 180 g), doses equal to or greater than 50 mg/kg slowed body weight gains.[64] Although overt clinical signs were not noted at 500 and 3000 mg/kg, these doses caused histopathologic degenerative changes in the liver and kidney.[64] In a 90-d subchronic study, doses of 1600 mg/kg for 60 d followed by 2500 mg/kg for another 30 d did not cause clinical signs or pathologic effects.[77] However, the actual data for that study were not presented, and because the report is inconsistent with the toxicity data from short-term (14 d) studies or even those with data from longer exposures, whether pathologic changes (for example, increased liver weight and hepatocellular hypertrophy) were, in fact, nonexistent is unclear.[64]

When lifetime toxicity–carcinogenicity exposures were started in utero and terminated at week 123 in Sprague–Dawley rats, doses of 135 mg/kg were associated with reduced body weight at euthanasia, decreased survival (25% versus 35%), and slightly increased incidence of hepatocellular carcinoma.[77] However, because the maximal tolerated dose (MTD) had been exceeded, the results from this group could not be used for assessment of carcinogenesis. According to current Food and Drug Administration guidelines, the main criteria for setting an MTD is that the animals remain in good clinical condition and do not lose or fail to gain body weight to an extent greater than 10% of similarly aged controls.[54] This definition allows subtle biochemical and cellular end points, such as induced mitogenesis, at the MTD. In lifetime studies, an FBZ dose of 45 mg/kg, which approximates the MTD, caused morphologic changes of hepatocellular hypertrophy and hyperplasia.[77] A pathology working group viewed the histopathologic changes as an adaptive response to toxicity unrelated to the formation of hepatic neoplasms. The group reached 2 other conclusions: 1) the lifetime no-observed adverse effect level for maternal and reproductive toxicity in rats was set at 15 mg/kg daily, and 2) doses higher than 15 mg/kg increase the incidence of hepatocellular altered foci and hypertrophy. Recent findings that administration of a 45 mg/kg dosage to rats for only 2 mo increased relative liver weights, caused periportal hepatocellular hypertrophy, and increased mitotic activity in rats led to the conclusion that this dosage triggers cell proliferation.[65] From the cited studies, the 45 mg/kg dosage likely was close to the MTD for carcinogenicity studies with rats, although no particular dosage has been accepted as such.

Mice appear to be less sensitive to FBZ than are rats. However, little species-specific information is available. A 2-y carcinogenicity study with doses as high as 405 mg/kg daily did not show an increase in tumor incidence.[77] The no-observed adverse effect level for mice was set at 135 mg/kg daily.[77]

Tumor Promoter Studies

As previously mentioned, lifetime studies in mice and rats indicate that FBZ itself is not a carcinogen. Histologic changes consisting of hepatocellular hypertrophy, bile duct proliferation, hyperplasia, and vacuolation occur in the livers of rats given at least 45 mg/kg.[77] The WHO joint expert committee viewed these changes as an adaptive response to toxicity; the question of whether FBZ could serve as a tumor promoter remained unresolved and was addressed later.[65,77] Dosages of 45 mg/kg or greater in rats likely achieved toxicity, and the histologic features of proliferation were secondary to chronic insult to the liver. Cells in chronically injured tissues are exposed continuously to endogenous mitogens (for example, growth factors), which ultimately promote cancer development through clonal expansion of already initiated cells.[50]

Carcinogenesis is a multistage continuous and dynamic process that is conceptually divided experimentally into the stages of initiation, promotion and progression. Therefore, even when not a complete carcinogen itself, a compound can contribute to cancer susceptibility by promoting proliferation of previously initiated cells. For example, compounds (such as dioxin), which cause no DNA damage and are negative by the Ames test, are potent tumor promoters.[40] Tumor promoters typically act over time, and what is initially a reversible lesion eventually develops into cancer after prolonged exposures to a promoter agent.

To resolve whether FBZ acts as a tumor promoter, a medium-term liver bioassay known to be a reliable test for the detection of carcinogens as well as promoters of hepatocarcinogenesis, was applied to FBZ and its metabolite oxfenbendazole (OXF).[43,63,65] The assay was a 2-stage liver carcinogenesis model in which rats were initiated with a single in vivo dose of the genotoxic hepatocarcinogen diethylnitrosamine; 1 wk later they began receiving a diet containing FBZ at 0, 70, 200, 600, 1800, and 3600 ppm for 8 wk. One of the biomarkers to assess promotion (decrease in gap-junctional intercellular communication connexin 32) appeared to indicate a positive effect at doses equal to or greater than 70 ppm, the other marker (glutathione S-transferase placental form, GST-P) was only positive at doses of or exceeding 1800 ppm.[65] Given these results, the authors proposed that FBZ has liver tumor-promoting activity similar to that of phenobarbital.

Because there are no standard criteria for classifying a compound as a promoter, the experimental conditions and type of biomarkers used are an important source of variation for this type of study. Of the numerous biochemical markers for early detection of preneoplastic cells, the number and areas of GST-P–positive liver foci undoubtedly have been the most widely used endpoints, because they correspond well with the incidence of hepatocellular carcinomas in long-term in vivo assays.[51,63] GST-P is an enzyme strongly expressed in so-called initiated cells, but not normal hepatocytes, during the early stage of chemically induced hepatocarcinogenesis.[58] By considering this assay as the hallmark to identify preneoplastic hepatic foci, we can state that FBZ seems to act in a manner similar to tumor promoters, although at clinically toxic doses (that is, doses exceeding the MTD). Therefore, the results of GST-P assays would not be noteworthy in terms of risk at therapeutic levels of FBZ.

This is not the case for the inhibition of connexin 32 by FBZ, which occurs at much lower and noncytotoxic levels within the therapeutic range for FBZ (70 to 200 ppm).[65] Decreased expression of connexin 32, the predominant hepatic gap junction protein, is shared by numerous tumor-promoter agents that exert their promoting activity through different mechanisms.[35,73] However and unlike the GST-P endpoint, the inhibition of

connexin 32 is a necessary but insufficient factor to consider a compound as a tumor promoter. Two things are necessary for an initiated cell to proliferate: a) an intracellular signal from the chemical to block contact inhibition and transfer of signals from cell to cell via gap junctions, and b) an intracellular signal to proliferate. Disruption of gap junctions does not necessarily imply that the chemical induces a mitogenic signal.

A previous study looked at another potential biomarker of tumor promotion: the induction of certain cytochromes P450 such as CYP2B1.[65] These are discussed later in the text. Nevertheless, that a compound exerts an inducing effect on cytochrome P450s may not necessarily imply a promoter or mitogenic action; and so, induction should not be considered a reliable endpoint of promoter activity.[37] This point is illustrated by the induction of CYP1A2 by FBZ in relation to a known dietary carcinogen, MeIQx (2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline). MeIQx is an heterocyclic amine thought to be metabolically bioactivated to genotoxic intermediates in the liver by CYP1A2.[36,74] Combining FBZ (600 ppm in the diet) with MeIQx did not enhance MeIQx-induced hepatocarcinogenesis, despite the fact that FBZ caused a 2.3-fold increase in CYP1A2 levels.[70] In this case, only GST-P positive liver cell foci were used to assess carcinogenicity.

When the same 2-stage carcinogenesis model was applied to OXF, which is the primary in vivo metabolite of FBZ, lower doses of OXF (10 and 100 ppm) had greater effects at inducing the same cytochromes and affecting the same assays (connexin 32 and GST-P) used as biomarkers of preneoplastic lesions.[43] The higher potency of OXF suggests that many of the in vivo effects of FBZ likely were caused through this metabolite.

In conclusion, the medium-term liver bioassay in rats showed that FBZ may act as a promoter when combined with certain genotoxic chemicals like diethylnitrosamine but not with other compounds, such as MeIQx. However, because no effects were seen in uninitiated groups, these studies also support previous evidence from long-term carcinogenicity experiments that FBZ alone is not a complete carcinogen.

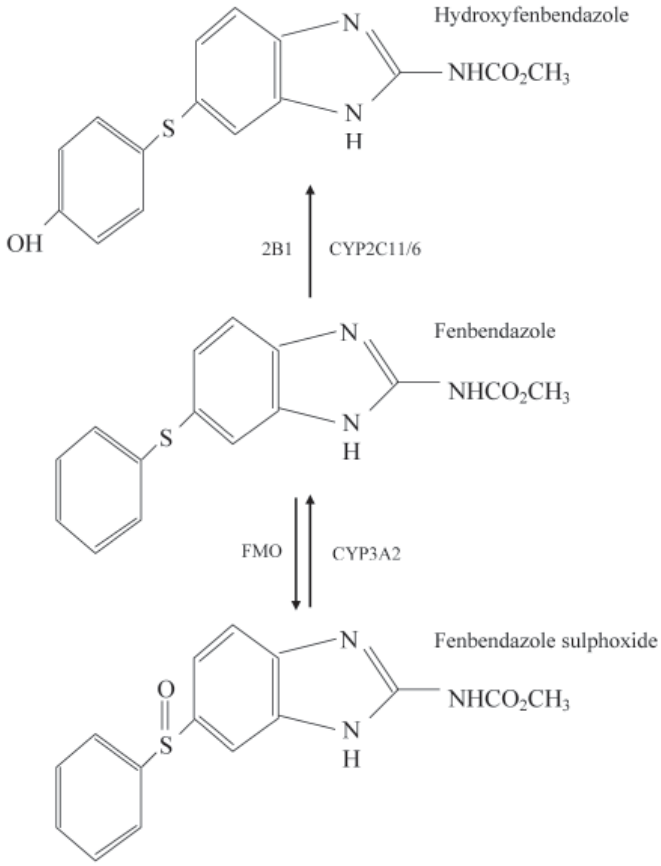

Figure 1. Initial FBZ biotransformation and key cytochrome isoforms implicated in rats. Total metabolite production toward FBZ sulphoxide (also called oxfenbendazole, OXF) and hydroxyfenbendazole is nearly identical in rats.[46,67] These are the 2 main metabolites detected in plasma and primarily are eliminated through bile into feces. Notice that fenbendazole and OXF are metabolically interconvertible. FMO, flavin monooxygenase system; CYP, cytochromes.

Effect on Cytochromes P450

The widespread use of FBZ in veterinary medicine has prompted numerous studies on its effect on hepatic biotransformation enzymes. FBZ is biotransformed largely by hepatic microsomal P450 and to a lesser extent by flavin monooxygenase enzyme systems (Figure 1).[46,66,67] Consequently, any changes in the activity of these enzymes may alter the pharmacokinetics of FBZ and other coadministered xenobiotics and divert their normal biotransforming pathways. In fact, goats pretreated with the nonspecific P450 inhibitor piperonyl butoxide showed more than 3-fold increases in the relative bioavailability (that is, area under the curve) of FBZ and its primary metabolite OXF.[6] Interestingly, coadministration of both drugs greatly potentiated their antinematodal activity, and this potentiation was attributed to the extended pharmacokinetic profile of FBZ.[6]

Early studies in rodents showed that FBZ did not exert either positive or negative effects on total microsomal P450, even at 100 mg/kg daily for 15 d (that is, at 10 times the therapeutic level).[12,44] No effect was also found in other microsomal constituents, including NADPH cytochrome c reductase, cytochrome b5, and glutathione S-transferase. These studies were conducted before the advent of more recent molecular biology techniques that have classified the P450 system into families, subfamilies and specific isoforms; so far, at least 93 functional cytochrome genes have been sequenced in mouse liver, with 82 members belonging to the 4 major drug-metabolizing families.[39] Table 1 provides further information illustrating the content and relative concentrations of the common P450 enzymes in rat hepatic microsomes.

FBZ induces 2 members of the highly conserved 1A subfamily (1A1 and 1A2) as well as cytochrome 2B1 in rats.[2,65] These isoforms are constitutively expressed at very low or virtually undetectable levels (Tables 1 and 2), which could explain the lack of effect on total P450 contents and associated enzyme activities in the early studies. The CYP1A1 and 1A2 protein levels in rat hepatocytes incubated with different concentrations of FBZ for 48 to 72 h increased 8-fold and 7-fold, respectively, along with a 2- to 3-fold increase in the levels of the corresponding functional markers, ethoxyresorufin O-deethylation (EROD) and methoxyresorufin O-demethylation (MROD).[2] Rats dosed with 200 ppm (that is, 1.3 times therapeutic levels) for 8 wk and euthanized 1 wk later had a 3-fold induction of CYP1A2, whereas CYP1A1 remained undetectable.[65] However, the magnitude of this induction caused by FBZ can be considered minor when compared to classic inducers like 3-methylcholanthrene and β-naphthoflavone, for which increases of several hundredfold are typical after 24 h of treatment (Table 2). Studies in primary cultures of rabbit hepatocytes have shown that, at least for CYP1A1, the mechanism of FBZ induction involves transcriptional activation of gene expression.[18]

From a practical standpoint, CYP1A1 and 1A2 have received much attention because they are well known for activating a number of procarcinogens, such as aromatic amines present in

Table 1. Concentrations of P450 enzymes in rat hepatic microsomes

	Specific content (pmol/mg protein)		% of total spectral P450	
P450 isoform	Study 1	Study 2	Study 1	Study 2
Total P450[a]	430 ± 60	1090 ± 120	100	100
Total isoforms[b]	299	556	69.5	51
CYP1A2	3.1 ± 1.2	not determined	0.7	not determined
CYP2A2	not determined	10 ± 6	not determined	1.0
CYP2B1	6.9 ± 1.7	11 ± 10	1.6	1.0
CYP2C11	139 ± 29	230 ± 25	32.3	21.1
CYP2D1	66 ± 7.7	not determined	15.3	
CYP2E1	not determined	82 ± 37	not determined	7.5
CYP3A2	84 ± 11	165 ± 17	19.5	15.1
CYP4A2	not determined	58 ± 4	not determined	5.3

Data deduced from references 47 (study 1) and 24 (study 2).
[a]Determined spectrally.
[b]Sum of enzymes determined immunochemically. Note that 30.5% (study 1) and 49% (study 2) of the other CYP isoforms were not determined.

organic pyrolysis products and polycyclic aromatic hydrocarbons present in tobacco smoke and charcoal-broiled meat.[62] Furthermore, a recent critical review provides strong evidence that the chemoprotective effect of numerous flavonoids present in fruits, vegetables, and plant beverages is, among other mechanisms, through inhibition of the metabolic activation of procarcinogens by cytochromes P450 1A1 and 1A2.[45] The cited review also describes in vivo studies showing that some flavonoids can suppress the tumor formation induced by polycyclic aromatic hydrocarbons and other carcinogens in experimental animals.[45] The wide application of genetic engineering and polymerase chain reaction techniques to better define the roles of specific cytochrome isoforms have revealed that overexpression of the *CYP1A1* gene is accompanied by alternative splicing variants of the enzyme that are expressed in compartments other than the endoplasmic reticulum (that is, the nucleus) and facilitates the neoplastic transformation of normal cells exposed to procarcinogens.[38]

Because liver microsomes from rodents have low expression of CYP1A2 and virtually undetectable levels of CYP1A1 and CYP2B1, their overall contribution to total biotransformation of most xenobiotics is probably small unless they became induced.[14,65] Of the 3 cytochromes that have been so far shown to be induced by FBZ, only CYP2B1 appears to participate in the first oxidation step for FBZ (Figure 1). In rats, the first 2 oxidations occur through CYP3A and the flavin-monooxygenase system, and CYP2C6/11 and CYP2B1 are involved in the conversion to the 4′-hydroxyl metabolite (Figure 1).[46,67] By inducing CYP2B1, FBZ may hasten the oxidation to the FBZ-OH metabolite and alter its own bioavailability; this scenario remains to be studied. Similarly, drug interactions from metabolism through these inducible FBZ isoforms may be discovered in the future. For example, a 40% lower plasma concentration and faster clearance of propanolol have largely been attributed to a 1.5- to 2-fold induction of CYP1A2 by ginkgo herbal extracts, although the involvement of other isoforms was not ruled out.[79]

In unpublished observations with mice, we found that FBZ is converted rapidly to OXF when injected intravenously at a dosage of 10 mg/kg. However, the rates of conversion differed markedly among animals, with concentrations of OXF measuring 25% to 400% of those attained for FBZ at 18 to 30 min after injection. Therefore, marked differences in expression levels of the cytochromes involved in the sulfoxidation of FBZ likely largely account for the extreme interanimal variation in biotransformation.

Bone Marrow Effects

In recent years, clinical observations of myelosuppression associated with FBZ treatment have been documented for various animal species including porcupines, canines, pigeons and doves, and tortoises; however, we found no similar reports for rodents.[17,22,26,48,76] A common denominator in these cases of myelosuppression was the diagnosis of bone marrow hypoplasia within a few days of initiating FBZ treatment. The dosages given were all in the upper end of the recommended range (50 to 100 mg/kg for several days) and at least for birds, a possible dose relationship was mentioned (that is, higher morbidity and mortality in birds given higher doses), together with a greater occurrence in columbiform birds.[22,26] However, whether myelosuppression develops seems to be unpredictable, and no animal models are available to reproduce the condition, suggesting an immune-mediated mechanism of action or an idiosyncratic reaction in extremely sensitive animals. An idiosyncratic reaction is possible because FBZ also inhibits mammalian microtubule assembly and blocks mitosis of human lymphocytes at metaphase.[13,25] A sensitization reaction that involves the immune system and does not have a dose–response relationship is also feasible because very high doses of FBZ that result in toxicosis affect organs (for example, liver, kidney) other than the hematopoietic system. The selective toxicity of benzimidazole anthelmintics toward nematodes is assumed to derive from the greater susceptibility of parasitic β tubulins (compared with that of their mammalian counterparts) to inhibition of polymerization. This susceptibility appears to correlate well with the affinity of the benzimidazole drugs for binding tubulin, to the extent that determining key amino acid residues in the structure of β tubulin can be used to predict resistance to benzimidazole drugs.[33,57] Rodents have been used to understand the mechanisms of drug-induced bone marrow failure for some compounds such as benzene and chloramphenicol; however, they may not be suitable animal models for FBZ because no myelosuppresive effects in rodents have been reported.[10]

Effects on the Immune System

Immunologic function is a critically important variable that underlies most, if not all, experimental protocols. Several studies have examined whether FBZ exerts any immunomodulatory effects on the immune system.[8,15,16,49,55] Some of these studies, together with the effects of other anthelmintics, have been re-

Table 2. Induction of liver P450 isoenzymes (CYP1A1 and 1A2) and the corresponding dealquilation assays (EROD and MROD) by FBZ, 3-methylcholanthrene (3MC), polychlorinated biphenyls (PCB), β-naphthoflavone (βNF), and phenobarbital (PB) in rats

Treatment	P4502B1 (pmol/mg protein)	P4501A1 (pmol/mg protein)	P4501A2 (pmol/mg protein)	EROD (nmol/min/mg)	MROD (nmol/min/mg)	Reference
Control	not determined	<1	16–35	0.19–0.30	0.06–0.21	74
3MC	not determined	550–720	520–740	11.8–13.9	0.91–0.21	74
PCB	not determined	1100	990	13.82 ± 2.62	2.30 ± 0.33	74
βNF	not determined	880	580	12.12 ± 0.24	0.43 ± 0.06	74
Control	6.9 ± 1.7	not determined	3.2 ± 1.2	not determined	not determined	47
PB	360 ± 21	not determined	2.3 ± 0.9	not determined	not determined	47
Control	<1	<1	6.7 ± 6.3	not determined	not determined	65
FBZ	36.2	<1	20.8 ± 5.5	not determined	not determined	65

Control animals received an intraperitoneal injection of corn oil or were untreated; experimental rats were given 3MC (40 mg/kg) or βNF (100 mg/kg) intraperitoneally for 3 d and were euthanized 24 h after the last treatment, one injection of PCB (Aroclor 1254; 500 mg/kg) intraperitoneally and were euthanized after 5 d, or PB (80 mg/kg) intraperitoneally for 4 d and euthanized 24 h after the last treatment. FBZ (200 ppm) was provided for 8 wk in the diet of diethylnitrosamine-initiated rats. EROD and MROD assays were done with a substrate concentration of 50 μM. The total cytochrome P450 content in the liver microsomes of control rats ranges between 400 and 1000 pmol/mg protein (Table 1).

viewed recently.[56] Both stimulatory and suppressive effects on different components of the immune system have been reported, adding to the difficulties of interpreting their physiologic implications. Nevertheless, with the use of genetically modified mice, it may now be possible to relate changes in specific components of the system to their physiologic implications to the animal. One study looked at the effects of FBZ on a mouse model (nonobese diabetic, NOD), where T cells become autoreactive against pancreatic islet antigens, thus causing type I diabetes.[16] This study found that exposure of NOD mice to a FBZ-medicated diet for 23 wk did not alter the incidence or onset of diabetes compared with that of the control group. Furthermore, no changes were seen in lymphocytes subpopulations (CD4:CD8) or T lymphocyte proliferative responses to Con A. Experiments in common strains of mice (BALB/CByJ, C57BL/6J) fed 100-ppm FBZ diets for 2 wk found no effect on a number of specific immune responses: ability to generate helper T cells, allospecific cytolytic T cells, priming of pre-killer cells, and production of specific antibodies against an influenza virus.[55] A recent retrospective study reported FBZ did modulate the inflammatory process in F344 rats.[29] During a study period, the rats were treated with FBZ as part of a colony management directive. The investigators found that the experimental rats, which were injected with LPS as part of the study, demonstrated increased weight loss, microglial activation, and loss of astrocytes.

Extensive studies highlighting the effects of FBZ on immune system function have been performed in sheep. These studies were conducted in nonparasitized lambs and examined multiple immune responses after the administration of FBZ or OFX.[8,49,69] Briefly, 6-mo-old lambs were drenched with a single dose of FBZ or OXF on days 0 and 28, and 1 d after each drench, they were injected with human erythrocytes and ovalbumin. The assessment of the immune system included T cell and B cell proliferation assays and antibody responses against the human erythrocytes and ovalbumin antigen injected. These studies showed that for some sampling dates, both B and T lymphocytes collected after the first and particularly after the second administration of FBZ or OXF had lower stimulation indices. In addition, antibody responses were depressed after the second antigen injection. The studies concluded that FBZ and OXF may affect the general ability of circulating lymphocytes to divide, particularly those in the secondary immune response. These findings are consistent with earlier reports demonstrating the ability of benzimidazole drugs, including FBZ, to block mitosis of human lymphocytes in culture.[25] However, the fact that the most noticeable effects occurred after a second challenge to FBZ also suggests an immune response in which the drug behaves as a hapten during the initial exposure.

These studies in sheep prompted similar experiments in rodents, which are underway in our laboratory. Recent work has demonstrated that aged (22-mo-old) BALB/c mice on FBZ treatment regimens have less B cell proliferation in response to mitogens than do young (3- to 4-mo-old) BALB/c mice.[78] Additional experiments to assess other aspects of the in vitro and in vivo immune responses during FBZ treatment are ongoing.

In conclusion, there are conflicting reports on whether FBZ exerts immunomodulatory actions. Although most studies have shown no effect of FBZ on selected immune responses, the question of whether FBZ suppresses lymphocyte proliferation remains controversial. Because the immune system is under continuous self-regulation to balance the intensity and specificity of its responses, any drug that suppresses lymphocyte proliferation may affect multiple responses such as allergy, autoimmunity, graft rejections in transplants, and antibody formation.

Reproduction, Teratologic, and Behavioral Studies

Effects on reproductive performance and offspring production can have dire consequences for researchers, especially when dealing with rare transgenic strains. Therefore, the reproductive, teratogenic, and behavioral effects of FBZ are critically important to understand. Reproductive studies conducted over 3-generation SD rats at doses of at least 5 mg/kg concluded that dosages of 45 mg/kg or greater caused reduced fertility and severe signs of toxicosis in pups (for example, decreased survival indices, decreased body weights at birth, slower lactational growth, and so forth).[77] Because the lower dosages of 5 and 15 mg/kg did not cause significant alterations, the no-observed-effect limit for reproductive effects was set at 15 mg/kg daily.[77] A recent report conducted from retrospective breeding records noted an association between litter size (that is, fecundity) and FBZ treatment in rats.[32] The rats that were given FBZ feed on a intermittent or continuous basis for as long as 7 wk had 3 to 4 fewer pups per litter than did nonmedicated animals. No other endpoints of reproductive toxicity were evaluated (for example, weaning and lactation indices, fertility, stillbirths), and several factors could have influenced the interpretation of the results, including the greater age of the dams on the medicated diet and the differing nutrient composition of 2 diets. In the same study, no effect on litter size was observed in genetically

Table 3. Biologic effects of FBZ at therapeutic levels (150 ppm; 8 to 12 mg/kg daily) in rodents and other species

		Mice	Rats	Other species	References
Effects on					
	Immune system				
				Decreased B and T proliferation in sheep	8, 49
				Decreased antibody responses to erythrocytes and ovoalbumin	8, 49
				Increased inflammation with treatment with LPS	29
	Reproduction				
			Decreased fecundity		32
	Carcinogenesis				
			Decreased amount of connexin 32 after initiation with diethylnitrosamine		65
	Bone marrow				
				Myelosuppression	26, 48, 76
	Cytochromes				
			Induction of CYPs 1A1, 1A2, 2B1, and 4A1		2, 65
				Induction of CYP1A2 in pigs	3, 59
				Induction of CYP1A1 in rabbits	18
No effects on					
	Immune system				
		Ability to: i) generate specific T helper cells			55
		ii) generate allospecific cytolytic T cells			55
		iii) prime for pre-killer cells			55
		iv) generate antibodies against influenza virus			55
		Onset and incidence of diabetes in NOD mice			16
	Reproduction				
			No effect at ≤5 mg/kg		77
	Carcinogenesis				
			Lack of promotion with MeIQx		70
		Lack of carcinogenesis	Lack of carcinogenesis		77
	Behavior				
		Food search, drinking, licking			5, 34

epilepsy-prone rats (GEPR, substrain 9) that received a similar regimen of FBZ treatment.

No evidence of teratogenicity was seen at levels as high as 2500 mg/kg in rats; mice were not studied.[77] In another study, the offspring of SD rats continuously exposed to therapeutic levels of FBZ were examined in a variety of behavioral teratologic paradigms.[5] Of 5 behaviors examined, 2 (negative geotaxis, digging maze performance) were unaffected, and 3 (delayed righting reflex, Morris water maze, and running wheel) showed subtle or minor alterations in performance. However, because the effects were subtle, their biologic relevance was questionable, and the overall conclusion was that FBZ had minimal consequences on behavioral and developmental studies. Although the cited study did not report the litter size to assess for fecundity, there was no effect on body weight gain in pups. Two additional studies also reported the lack of behavioral effects in rats on FBZ-medicated diets.[34,72] In those studies, standard tasks included food search, drinking behavior, and lick rates.

Conclusions

A review of the literature has shown that FBZ at therapeutic levels will not cause any toxic effects but, like any other drug, it has some physiologic actions that potentially can change the outcome of laboratory experiments (Table 3). Although long-term carcinogenicity experiments have proven that FBZ itself is not a complete carcinogen, FBZ given at therapeutic levels after certain genotoxic initiators like diethylnitrosamine did inhibit gap junction intercellular communication, which is a common

biomarker used to detect promoter activity. However, histologic changes compatible with those of promoter activity were seen only at doses exceeding the therapeutic level. In addition, in vivo and in vitro evidence from rodents and other animal species indicates that, compared with prototype inducers, FBZ moderately induces cytochromes 1A1, 1A2, and 2B1. These isoforms (particularly 1A1) play an important role in activating endogenous (for example, estrogens) and exogenous procarcinogens. The effects of potential pharmacokinetic interactions of other drugs with FBZ, including acceleration of its own CYP2B1-mediated oxidation to hydroxyl FBZ, have not been studied. At present, there are conflicting reports on whether FBZ exerts immunomodulatory actions, and although most studies have not shown any effects on selected immune responses, whether lymphocyte proliferation in vitro is suppressed remains controversial and deserves further consideration. At therapeutic levels, neither reproductive, teratologic, nor behavioral studies have shown any significant biologic effects of FBZ.

References

1. **Agersborg SS, Garza KM, Tung KSK.** 2001. Intestinal parasitism terminates self tolerance and enhances neonatal induction of autoimmune disease and memory. Eur J Immunol **31:**851–859.
2. **Baliharova V, Skalova L, Mass RFM, De Vrieze G, Bull S, Fink-Gremmels J.** 2003. The effects of benzimidazole anthelmintics on P4501A in rat hepatocytes and HepG2 cells. Res Vet Med **75:**61–69.
3. **Baliharova V, Velik J, Šavlik M, Szotakova B, LAmka J, Tahotna L, Skalova L.** 2004. The effects of fenbendazole, flubendazole and mebendazole on activities of hepatic cytochromes P450 in pig. J Vet Pharmacol Therap **27:**85–90.
4. **Barlow SC, Brown MM, Price HV.** 2005. Eradication of *Syphacia muris* from food-restricted rats without environmental decontamination. Contemp Top Lab Anim Sci **44:**23–25.
5. **Barron S, Baseheart BJ, Segar TM, Deveraux T, Willford JA.** 2000. The behavioral teratogenic potential of fenbendazole: a medication for pinworm infestation. Neurotoxicol Teratol **22:**871–877.
6. **Benchaoui HA, and McKellar QA.** 1996. Interaction between fenbendazole and piperonyl butoxide: pharmacokinetic and pharmacodynamic implications. J Pharm Pharmacol **48:**753–759.
7. **Bugarski D, Jovcic G, Katic-Radivojevic S, Petakov M, Krstic A, Stojanovic N, Milenkovic P.** 2006. Hematopoietic changes and altered reactivity of IL-17 in *Syphacia obvelata*-infected mice. Parasitol Int **55:**91–97.
8. **Cabaj W, Stankiewicz M, Jonas WE, Moore LG.** 1994. Fenbendazole and its effect on the immune system of the sheep. N Z Vet J **42:**216–220.
9. **Campbell WC.** 1990. Benzimidazoles: veterinary uses. Parasitol Today **6:**130–133.
10. **Chen J.** 2005. Animal models for acquired bone marrow failure syndromes. Clin Med Res **3:**102–108.
11. **Coghlan LG, Lee DR, Psencik B, Weiss D.** 1993. Practical and effective eradication of pinworms (*Syphacia muris*) in rats by use of fenbendazole. Lab Anim Sci **43:**481–486.
12. **Dalvi RR, Gawai KR, Dalvi PS.** 1991. Lack of in vivo and in vitro effects of fenbendazole on phase I and phase II biotransformation enzymes in rats, mice and chickens. Vet Hum Toxicol **33:**548–551.
13. **Dawson PJ, Gutterdge WE, Gull K.** 1984. A comparison of the interaction of anthelmintic benzimidazoles with tubulin isolated from mammalian tissue and the parasitic nematode Ascadidia Galli. Biochem Pharmacol **33:**1069–1074.
14. **Dey A, Jones JE, Nebert DW.** 1999. Tissue and cell type-specific expression of cytochrome P450 1A1 and cytochrome P450 1A2 mRNA in the mouse localized in situ hybridization. Biochem Pharmacol **58:**525–537.
15. **Dvoroznakova E, Boroskova Z, Dubinsky P, Velebny S, Tomasovicova O, Machnicka B.** 1998. Changes in cellular immunity of mice treated for larval toxocarosis with fenbendazole. Helminthologia **35:**189–195.
16. **Franke DDH, Shirwan H.** 2006. Prophylactic fenbendazole therapy does not affect the incidence and onset of type 1 diabetes in nonobese diabetic mice. Int Immunol **18:**453–458.
17. **Gary AT, Kerl ME, Wiedmeyer CE, Turnquist SE, Cohn LA.** 2004. Bone marrow hypoplasia associated with fenbendazole administration in a dog. J Am Anim Hosp Assoc **40:**224–229.
18. **Gleizes-Escala C, Lesca P, Larrieu G, Dupuy J, Pineau T, Galtier P.** 1996. Effect of exposure of rabbit hepatocytes to sulfur-containing anthelmintics (oxfenbendazole and fenbendazole) on cytochrome P4501A1 expression. Toxicol In Vitro **10:**129–139.
19. **Goldenthal EI.** 1980. Three-generation reproduction study in rats. Mattawan (MI): International Research and Development Corporation.
20. **Godenthal EI.** 1980. 24-month oral carcinogenicity study in mice. Mattawan (MI): International Research and Development Corporation.
21. **Godenthal EI.** 1980. Lifetime oral toxicity study in rats. Mattawan (MI): International Research and Development Corporation.
22. **Gozalo AS, Schwiebert RS, Lawson GW.** 2006. Mortality associated with fenbendazole administration in pigeons (*Columba livia*). J Am Assoc Lab Anim Sci **45:**63–66.
23. **Hill WA, Randolph MM, Lokey SJ, Hayes E, Boyd KL, Mandrell TD.** 2006. Efficacy and safety of topical selamectin to eradicate pinworm (*Syphacia spp.*) infections in rats (*Rattus norvegicus*) and mice (*Mus musculus*). J Am Assoc Lab Anim Sci **45:**23–26.
24. **Hiroi T, Miyazaki Y, Kobayashi Y, Imaoka S, Funae Y.** 1995. Induction of hepatic P450s in rat by essential wood and leaf oils. Xenobiotica **25:**457–467.
25. **Holden HE, Crider PA, Wahrenburg MG.** 1980. Mitotic arrest by benzimidazole analogs in human lymphocytes cultures. Environ Mutagen **2:**67–73.
26. **Howard LL, Papendick R, Stalis IH, Allen JL, Sutheland-Smith M, Zuba JR, Ward DL, Rideout BA.** 2002. Fenbendazole and albendazole toxicity in pigeons and doves. J Avian Med Surg **16:**203–210.
27. **Huerkamp MJ, Benjamin KA, Webb SK, Pullium JK.** 2004. Long-term results of dietary fenbendazole to eradicate *Syphacia muris* from rat colonies. Contemp Top Lab Anim Sci **43:**35–36.
28. **Huerkamp MJ, Kimberley AB, Zitzow LA, Pullium JK, Lloyd JA, Thompson WD, Webb SK, Lehner NDM.** 2000. Fenbendazole treatment without environmental decontamination eradicates *Syphacia muris* from all rats in a large, complex research institution. Contemp Top Lab Anim Sci **39:**9–12.
29. **Hunter RL, Dong-Young C, Kincer JF, Cass WA, Bing G, Gash DM.** 2007. Fenbendazole treatment may influence lipopolysaccharide effects in rat brain. Comp Med **57:**487-492.
30. **Jackson TA, Hall JE, Boivin GP.** 1998. Ivermectin toxicity in multiple mouse lines. Lab Anim Pract **31:**37–41.
31. **Jacoby RO, Lindsey JR.** 1997. Health care for research animals is essential and affordable. FASEB J **11:**609–614.
32. **Johnston NA, Bieszcak JR, Verhulst S, Disney KE, Montgomery KE, Toth LA.** 2006. Fenbendazole treatment and litter size in rats. J Am Assoc Lab Anim Sci **45:**35–39.
33. **Katiyar SK, Gordon VR, McLaughlin GL, Edlind TD.** 1994. Antiprotozoal activities of benzimidazoles and correlations with β-tubulin sequence. Antimicrob Agents Chemother **38:**2086–2090.
34. **Keen RG, Macinnis MLM, Guilhardi P, Chamberland KA, Church RM.** 2005. The lack of behavioral effects of fenbendazole: a medication for pinworms infection. Contemp Top Lab Anim Sci **44:**17–23.
35. **Krutovskikh VA, Mesnil M, Mazzoleni G, Yamasaki H.** 1995. Inhibition of rat liver gap junction intercellular communication by tumor-promoting agents in vivo. Lab Invest **72:**571–577.
36. **Kuribayashi M, Asamoto M, Suzuki S, Hokaiwado N, Ogawa K, and Shirai T.** 2006. Lack of modification of 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) rat hepatocarcinogenesis by caffeine, a CYP1A2 inducer, points to complex counteracting influences. Cancer Lett **232:**289–299.

37. **Lake BG, Renwick AB, Cunninghame ME, Price RJ, Surry D, Evans DC.** 1998. Comparison of the effects of some CYP3A and other enzyme inducers on replicative DNA synthesis and cytochrome P450 isoforms in rat liver. Toxicology **131:**9–20.
38. **Leung YK, Lau KM, Mobley J, Jiang Z, Ho SM.** 2005. Overexpression of cytochrome P450 1A1 and its novel spliced variant in ovarian cancer cells: alternative subcellular enzyme compartmentation may contribute to carcinogenesis. Cancer Res **65:**3726–3734.
39. **Löfgren S, Habgjork AL, Ekman S, Fransson-steen R, Terelius Y.** 2004. Metabolism of human cytochrome P450 marker substrates in mouse: a strain and gender comparison. Xenobiotica **34:**811–834.
40. **Mandal PK.** 2005. Dioxin: a review of its environmental effects and its aryl hydrocarbon receptor biology. J Comp Physiol B **175:**221–230.
41. **McNair DM, Timmons EH.** 1977. Effects of *Aspiculuris tetraptera* and *Syphacia obvelata* on exploratory behavior of an inbred mouse strain. Lab Anim Sci **27:**38–42.
42. **Michels C, Goyal P, Nieuwenhuizen N, Brombacher F.** 2006. Infection with *Syphacia obvelata* (pinworm) induces protective Th2 immune responses and influences ovoalbumin-induced allergic reactions. Infect Immun **74:**5926–5932.
43. **Mitsumori K, Onodera H, Shoda T, Uneyama C, Imazawa T, Takegawa K, Yasuhara K, Watanabe T, and Takahashi M.** 1997. Liver tumor-promoting effects of oxfenbendazole in rats. Food Chem Toxicol **35:**799–806.
44. **Mohn G, Philipp EM.** 1981. Effects of *Syphacia muris* and the anthelmintic fenbendazole on the microsomal monooxygenase system in mouse liver. Lab Anim **15:**89–95.
45. **Moon YJ, Wang X, Morris ME.** 2006. Dietary flavonoids: effects of xenobiotic and carcinogen metabolism. Toxicol In Vitro **20:**187–210.
46. **Murray M, Hudson AM, Yassa V.** 1992. Hepatic microsomal metabolism of the anthelmintic benzimidazole fenbendazole: enhanced inhibition of cytochrome P450 reactions by oxidized metabolites of the drug. Chem Res Toxicol **5:**60–66.
47. **Nakamato T, Oda Y, Imaoka S, Funae Y, Fujimori M.** 1997. Effect of phenobarbital on the pharmacokinetics of lidocaine monoethylglycinexylidide and 3-hydroxylidocaine in the rat: correlation with P450 isoform levels. Drug Metab Disp **25:**296–300.
48. **Neiffer DL, Lydick D, Burks K, Doherty D.** 2005. Hematologic and plasma biochemical changes associated with fenbendazole administration in Hermann's tortoises (*Testudo hermanni*). J Zoo Wildlife Med **36:**661–672.
49. **Parish SJ, McFarlane RG, Familton AS, Abell TJ.** 1996. The effect of fenbendazole on the immune system of lambs. Proc New Zealand Soc Anim Prod **56:**80–83.
50. **Philip M, Rowley DA, Schreiber H.** 2004. Inflammation as a tumor promoter in cancer induction. Semin Cancer Biol **14:**433–439.
51. **Pitot HC, Dragan Y, Sargent L, Xu YH.** 1991. Biochemical markers associated with the stages of promotion and progression during hepatocarcinogenesis in the rat. Environ Health Perspect **93:**181–189.
52. **Plumb DC.** 1999. Veterinary drug handbook, 3rd ed. Ames (IA): Iowa State University Press.
53. **Pritchett KR, Johnston NA.** 2002. A review of treatments for the eradication of pinworm infections from laboratory rodent colonies. Contemp Top Lab Anim Sci **41:** 36–46.
54. **Redbook 2000** [Internet]. Toxicological principles for the safety assessment of food ingredients: 2007 update [cited 26 Sep 2007]. Available at http://www.cfsan.fda.gov/~redbook/red-ivc6.html.
55. **Reiss CS, Herrman JM, Hopkins RE.** 1987. Effect of anthelminthic treatment on the immune response of mice. Lab Anim Sci **37:**773–775.
56. **Sajid MS, Muhammad ZIG, Iqbal MU.** 2006. Immunomodulatory effect of various anti-parasitics: a review. Parasitology **132:**301–313.
57. **Samson-Himmelstjerna G, Witzendorff C, Sievers G, Schnieder T.** 2002. Comparative use of faecal egg count reduction test, egg hatch assay, and beta-tubulin codon 200 genotyping in small strongyles (cyathostominae) before and after benzimidazole treatment. Vet Parasitol **108:**227–235.
58. **Sato K.** 1988. Glutathione-S-transferases and hepatocarcinogenesis. Jpn J Cancer Res **79:**556–572.
59. **Savlík M, Fimanova K, Szotakova B, Lamka J, Skalova L.** 2006. Modulation of porcine biotransformation enzymes by anthelmintic therapy with fenbendazole and flubendazole. Res Vet Med **80:**267–274.
60. **Scholz H and Schultes E.** 1973a. Report on an acute oral safety evaluation of the anthelmintic HOE 881 in mice. Frankfurt am Main (Germany): Hoechst-Roussel.
61. **Scholz H and Schultes E.** 1973b. Report on an acute oral safety evaluation of the anthelmintic HOE 881 in rats. Frankfurt am Main (Germany): Hoechst-Roussel.
62. **Shimada T, Oda Y, Gillan EM, Guengerich P, Inoue K.** 2001. Metabolic activation of polycyclic aromatic hydrocarbons and other procarcinogens by cytochromes P4501A1 and P4501B1 allelic variants and other human cytochromes P450 in *Salmonella typhimurium* NM2009. Drug Metab Disp **29:**1176–1182.
63. **Shirai T.** 1997. A medium-term rat liver bioassay as a rapid in vivo test for carcinogenic potential: a historical review of model development and summary of results from 291 tests. Toxicol Pathol **25:**453–460.
64. **Shi-Xin X, Ding Z, Yu-Mei S, Shu-Huai W, Li-Qing S.** 1992. Subchronic toxicity studies of fenbendazole in rats. Vet Hum Toxicol **34:**411–413.
65. **Shoda T, Onodera H, Takeda M, Uneyama C, Imazawa T, Takegawa K, Yasuhara K, Watanabe T, Hirose M, Mitsumori K.** 1999. Tumor promoting effects of fenbendazole in rats. Toxicol Pathol **27:**553–562.
66. **Short CR, Barker SA, Hsieh LC, Ou SP, McDowell T.** 1988. Disposition of fenbendazole in the rabbit. Res Vet Sci **44:**215–219.
67. **Short CR, Flory W, Hsieh LC, Barker SA.** 1988. The oxidative metabolism of fenbendazole: a comparative study. J Vet Pharmacol Therap **11:**50–55.
68. **Silveira AC, Gilioli R, Oliveira ES, Bassani RA.** 2002. Subsensitivity to beta-adrenergic stimulation in atria from rats infested with *Syphacia sp*. Lab Anim **37:**63–67.
69. **Stankiewicz M, Cabaj W, Jonas WE, Moore LG, Chie WNG.** 1994. Oxfenbendazole treatment of non-parasitized lambs and its effect on the immune system. Vet Res Commun **18:**7–18.
70. **Suzuki S, Takahashi S, Asamoto K, Inaguma S, Ogiso T, Hirose M, Shirai T.** 2002. Lack of modification of 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx)-induced hepatocarcinogenesis in rats by fenbendazole – a CYP1A2 inducer. Cancer Lett **185:**39–45.
71. **Taffs LF.** 1976. Pinworm infections in laboratory rodents: a review. Lab Anim **10:** 1–13.
72. **Toth LA, Oberbeck C, Straign CM, Frazier S, Rehg JE.** 2000. Toxicity evaluation of phophylactic treatments for mites and pinworms in mice. Contemp Topics Lab Anim Sci **39:**18–21.
73. **Trosko JE, Ruch RJ.** 2002. Gap junctions as targets for cancer chemoprevention and chemotherapy. Curr Drug Targets **3:**1–17
74. **Turesky RJ, Constable A, Richoz J, Varga N, Markovic J, Martin MV, Guengerich FP.** 1998. Activation of heterocyclic aromatic amines by rat and human liver microsomes and by purified rat and human cytochrome P4501A2. Chem Res Toxicol **11:**925–936.
75. **Wagner M.** 1988. The effect of infection with the pinworm (*Syphacia muris*) on rat growth. Lab Anim Sci **38:**476–478.
76. **Weber MA, Miller MA, Neiffer DL, Terrell SP.** 2006. Presumptive fenbendazole toxicosis in North Amercian porcupines. J Am Vet Med Assoc **228:**1240–1242.
77. **World Health Organization** [Internet]. WHO food additive series, no. 29. Toxicological evaluation of certain veterinary drug residues: 1991 [cited 26 Sep 2007]. Available at http://www.inchem.org/documents/jecfa/jecmono/v29je01.htm.
78. **Zaias, J.** 2007. Personnel communication.
79. **Zhao LZ, Chen J, Ee PLR, Chan E, Duan W, Guan YY, Hong YH, Chen X, Zhou S.** 2006. Induction of propanolol metabolism by ginkgo biloba extract EGb 761 in rats. Curr Drug Metab **7:**577–587.