Office of Environmental Health Hazard Assessment

Joan E. Denton, Ph.D., Director
Headquarters • 1001 I Street • Sacramento, California 95814
Mailing Address: P.O. Box 4010 • Sacramento, California 95812-4010
Oakland Office • Mailing Address: 1515 Clay Street, 16th Floor • Oakland, California 94612

Agency Secretary

Arnold Schwarzenegger
Governor

M E M O R A N D U M

TO: Gary Patterson, Ph.D., Chief
Medical Toxicology Branch
Department of Pesticide Regulation
P.O. Box 4015
Sacramento, California 95812-4015

FROM: Anna M. Fan, Ph.D., Chief
Pesticide and Environmental Toxicology Section
1515 Clay Street, 16th Floor
Oakland, California 94612

DATE: XXXXX, 2000

SUBJECT: COMMENTS ON THE DRAFT RISK CHARACTERIZATION DOCUMENT FOR THE ACTIVE INGREDIENT THIABENDAZOLE PREPARED BY THE DEPARTMENT OF PESTICIDE REGULATION

Thank you for the opportunity to review the draft risk characterization document (RCD) for thiabendazole prepared by the Department of Pesticide Regulation (DPR). The Office of Environmental Health Hazard Assessment (OEHHA) reviews risk assessments prepared by DPR under the general authority of the Health and Safety Code, Section 59004, and also under the Food and Agricultural Code (FAC), Section 13129, in which OEHHA has the authority to provide advice, consultation, and recommendations to DPR concerning the risks to human health associated with exposure to pesticide active ingredients.

OEHHA's Introductory Comments:

1. We recommend providing further scientific support for the selection of the no-observed-adverse-effect-levels (NOAELs) used in the draft RCD. This especially applies to the chronic NOAEL, which appears to actually be a lowest-observed-adverse-effect-leveI (LOAEL) based on the cellular changes observed in animals at this level compared to controls.

2. We recommend reorganizing and expanding the description of human health effects from human exposure to TBZ, including a summary of the adverse effects of using TBZ as an anthelminthic drug.

3. We recommend including more discussion regarding dermal sensitization of TBZ in humans. The negative results from animal tests for dermal irritation and sensitization should be compared with the observation of skin rashes caused by TBZ in humans. The apparent contradiction in results might be related to study design or differences in susceptibility.

4. The potential for distributing TBZ into milk should be addressed in the section on "Pharmacokinetics." We also recommend including a discussion on the toxicity of TBZ metabolites and their role in the overall toxicological response from exposure to the parent compound.

5. We recommend expanding the discussion of the oncogenic potential of TBZ by addressing the significance of the positive genotoxic effects in support of a non-threshold approach to risk assessment, and to include all sites of tumor incidences in rats. A discussion of the structure-activity relationships for oncogenic activity among benzimidazole compounds would also be informative.

6. We recommend including more discussion of the mutagenic and clastogenic effects of TBZ, including its potential for aneugenicity.

7. The draft RCD concludes that children are more sensitive to TBZ exposure than adults based on pre- and post-natal and endocrine effects in animal studies. The draft RCD also states that margins of exposure (MOEs) for both daily and annual exposures to farm workers handling TBZ, and to the general public exposed via dietary consumption, were considered health protective. The draft RCD should include more discussion on whether the MOE calculations sufficiently account for the greater susceptibility of children.

8. The draft RCD concludes that children are more sensitive to TBZ exposure than adults. In the draft RCD, tolerances were assessed quantitatively without application of an additional uncertainty factor for a greater susceptibility of children and infants to TBZ

toxicity that appears to be warranted based on the toxicological data. Most of the current existing tolerances may not be health protective for children since they do not take into account their greater sensitivity to this chemical as compared with adults. This is an important issue and we feel that the incorporation of an additional uncertainty factor in the RCD to protect children and infants requires further discussion and consideration.

9. We recommend expanding the "Risk Characterization" section of the document to include a discussion of the uncertainties specific to the quality of the existing toxicological database. This section is also deficient in discussing other toxicological effects of TBZ for which risk assessment methodology is not yet accepted. These include the direct and indirect evidence of immunotoxicity and neurotoxicity of TBZ. The discussion on exposure issues related to more susceptible subpopulations should be expanded, including the identification of the unborn fetus, children and infants, woman of child-bearing age, and people with liver dysfunction as susceptible populations.

General Comments

1. More discussion is needed in the draft RCD to provide adequate scientific support for the selection of the no-observed-adverse-effect-leveI (NOAEL) of 10 mg/kg-day for chronic exposure. At this dose level there were two types of histopathological changes noted: gallbladder epithelial vacuolation and gallbladder inspissation (drying and/or thickening by the evaporation of readily vaporizable parts) (page 16). "The individual data from both sexes indicated an increase in the severity of epithelial vacuolation but not inspissation over the dose range tested." Neither of these toxicological effects was considered in the draft RCD as a NOAEL for use in risk assessment. We agree that the gallbladder inspissation may not be appropriate as a NOAEL because the individual data do not demonstrate a clear dose-response relationship. However, the individual data from both sexes indicate an increase in the severity of epithelial vacuolation. The reason given in the draft RCD (page 16, first paragraph) for discounting gallbladder epithelial vacuolation is "The physiological significance of the gall bladder epithelial vacuolation was not clear to either the study's pathologist, or Dr. Swenberg at ClIT (personal communication)." However, "vacuolation of the bile duct and distal tubules of the kidney" was deemed appropriate for the selection of the NOAEL (page 26, fourth paragraph). This inconsistent approach and use of the data to determine the biological significance of cellular vacuolation needs further clarification and support. (Note: Dr. Swenberg is identified as being at CIIT; however, is now on the faculty at the University of North Carolina. While it is possible that he provided his comments via personal communication while still at ClIT, more likely his affiliation should be identified as UNC.)

2. We identify 10 mg/kg-day as a lowest-observed-adverse-effect-leveI (LOAEL) based on the occurrence of gallbladder epithelial vacuolation. Therefore, we recommend that a LOAEL of 10 mg/kg-day be used in assessing risk for chronic TBZ exposure instead of its current identification and use as a NOAEL in the draft RCD. The biological significance of cellular vacuolation is well-established. There are numerous reports of

cellular vacuolation induced both in cultured mammalian cells and in experimental animals (Hirano et al., 1992; Plopper et al., 1992; Li et al., 1993; Muir et al., 1994; Reindel et al., 1994; Roy et al., 1998a; Roy et al., 1998b; Hertle et al., 1999; Kim et al., 1999). In every case referenced above, vacuolation is associated with cellular stress or cell killing. Therefore, vacuolation is often a sign of serious perturbation to cellular homeostasis.

3. Values for NOAELs from acute, subchronic, and chronic toxicity studies selected as the basis for risk assessment usually are inversely correlated with the length of exposure (i.e., the longer the exposure, the lower the value). This does not seem to be the case for TBZ as presented in the draft RCD. The NOAEL of 3.3 mg/kg-day, based on an oral human study, was selected for the evaluation of acute occupational and dietary exposures to TBZ (page 10, third paragraph), and the NOAEL of 10 mg/kg-day was selected for chronic exposure assessments (page 16, first paragraph). The latter was based on hepato/biliary toxicity in dogs and centrilobular hypertrophy in rats. If the value of 10 mg/kg-day were considered a LOAEL rather than a NOAEL per our recommendation (see comment number one above), then the values used for risk assessment would be inversely correlated with the length of exposure. If the draft RCD is not revised to include OEHHA's recommendation, then this dose-response anomaly requires a better explanation as well as a discussion of the implications for protection of worker and community health.

Dermal irritation and sensitization

Dermal irritation and sensitization is addressed in Appendix A prepared by the Worker Health and Safety Branch of the Department of Pesticide Regulation (DPR, page 28). Neither dermal irritation nor sensitization was addressed in the text of the draft RCD. We recommend that the RCD section on acute toxicity (page 9) be expanded to include a discussion of the potential for dermal irritation and sensitization. An explanation should be provided for the negative results in animal tests for dermal irritation and sensitization, considering the skin rashes caused by TBZ in humans (described in paragraph 3). The latter human effects and blood changes (anemia) observed in long-term toxicity studies in rats and dogs are suggestive of a potential for TBZ to cause immunotoxicity.

Human health effects

1. Human health effects from exposure to TBZ are described in the draft document under "Illness Reports (page 4), "Acute Toxicity" (pages 9 to 10), and "Subchronic Toxicity" (page 12). To give a more coherent discussion of human health effects, we recommend that these various descriptions of human health study results be separated from the animal studies and summarized in a separate section.

2. We recommend including a summary of the health effects information of TBZ when it is used as an anthelminthic (treatment for worms). Side effects frequently encountered

are anorexia, nausea, vomiting, and dizziness. Less frequently, diarrhea, weariness, drowsiness, giddiness, and headache occur. Occasional fever, rashes, erythema multiforme, hallucinations, sensory disturbances and Stevens-John syndrome (severe form of skin disorder with the involvement of oral-nasal and anogenital mucosa, the eyes, and viscera; can be fatal)-have been reported. Angioneurotic edema, shock, tinnitus, convulsions, and intrahepatic cholestasis are rare complications of the therapy.

Crystalluria with hematuria has been reported on occasion (Gilman, 1990). Side effects that are experienced rarely include tinnitus, collapse, abnormal sensation in the eyes, numbness, hyperglycemia, xanthopsia, enuresis, decrease in pulse rate and systolic blood pressure, and transitory changes in liver function (Gilman, 1980). TBZ used as an anthelminthic has been shown to produce striking effects on the immune system, apparently independent of its antiparasitic activities. It caused significant reduction of lung granulomas around Schistoma Mansom eggs, and footpad edema in response to schistosome egg antigens in unsensitized animals when given daily for eight days (Hewlett et al., 198 1). TBZ also produced a marked increase in the number of large, mitotically active lymphoblasts in the thymus cortex. In a companion study, TBZ administered together with dinitrofluorobenzene stimulated T cells in lymph nodes and spleen (Donskaya et al., 1982).

Oncogenicity

I . We recommend that the discussion on oncogenicity be expanded in the RCD to address data in support of a non-threshold approach, including the structure-activity relationship issue for oncogenic potential among other benzimidazoles. Such a discussion should clearly describe the complexity and uncertainties related to the mechanism of oncogenic activity of TBZ. However, TBZ has also been reported to damage DNA, measured as both the induction of sister-chromatid exchanges (SCEs) (de Pargament et al., 1987) and positive results in the comet assay (Sasaki et al., 1997). It is important to emphasize that TBZ has tested positive for genotoxicity in four independent in vivo studies, namely inducing SCEs in two separate in vivo studies in male mice (de Pargament et al., 1987; Ardito et al., 1996), micronuclei in one in vivo study in CFW mice, and DNA strand breaks, as assayed by the comet test using cells from mice exposed to TBZ in vivo (Sasaki et al., 1997). Generally speaking, positive evidence of genotoxicity in vivo is given more weight than evidence solely from in vitro tests. The replication of the positive findings in the SCE assay increases confidence in these results. Increased concern over the genotoxic activity of TBZ also comes from observations of positive findings in systems testing for different genotoxic endpoints.

2. TBZ induces adenomas of the thyroid follicular cells in male but not female Sprague-Dawley rats. It is not oncogenic in mice. Some evidence supports that the development of the tumors is secondary to hepatotoxicity. With regard to the discussion of the thyroid tumors observed in the Sprague-Dawley rats, it is helpful to refer directly to the U.S. Environmental Protection Agency's (U.S. EPA) science policy guidance on thyroid follicular cell tumors (Assessment on Thyroid Follicular Cell Tumors, U.S. EPA, 1998).

In this science policy document, U.S. EPA uses the term "mutagenic" in a broad sense. Examples taken from U.S. EPA's document include: "Mutagenic influences are evaluated by short-term tests for gene and structural chromosome mutations and other tests" (page 3); "Tumors seeming to arise from relevant mutagenic influences (e.g., gene mutations and structural chromosomal aberrations)" (page 17); and "Finally, careful review is warranted when both antithyroid and other determinants seem to apply to the observed thyroid tumors, such as when there are certain mutagenic influences (e.g., structural chromosome aberrations)." (page 28). Clearly, TBZ should be regarded as having mutagenic effects, for the purposes of determining which dose-response methodology is appropriate for evaluating the thyroid follicular cell tumor response under U.S. EPA's science policy guidance. In the case of TBZ, where there is evidence for both mutagenic and antithyroid effects, U.S. EPA's guidance recommends that both a linear and a margin of exposure approach be used in assessing the dose-response.

3. The draft RCD states "In summary, the weight of evidence does not indicate that the oncogenic potential of thiabendazole should be considered for linear response analysis. 1) Thiabendazole was genotoxic, but the genotoxic effects of thiabendazole were likely related to spindle disruption. Spindle inhibitors are known to have a threshold." The statement that spindle inhibitors are known to have a "threshold" does not represent a widely accepted viewpoint within the scientific community, and should be removed. Moreover, not all of the genotoxic effects of TBZ are related to spindle disruption. TBZ has been reported to induce mutations in Salmonella (Zeiger et al., 1988), positive results in vivo in the comet assay (indicative of DNA strand breaks), and SCEs in vivo in two independent experiments. None of these endpoints can be ascribed to spindle disruption.

4. The discussion of the oncogenic endpoints should be expanded to cover additional sites observed in the F344 rat bioassays. Preputial gland adenoma increased to a statistically significant extent in two separate studies in male F344 rats. Increased tumor incidence was observed in the high-dose groups, which experienced large reductions in body weight gain (i.e., 40 percent reduction). Clitoral gland adenoma increased in two separate studies in female F344 rats. In one study the increase was statistically significant. Increased tumor incidence was observed in the high-dose groups, which experienced large reductions in body weight gain (i.e., 40 percent reduction). The two series of studies conducted with F344 rats were not submitted to DPR, but were published in the scientific literature (Hayashida et al., 1985; Fuji et al., 1991). The RCD could provide more detail on the F344 rat studies. For example, it would be helpful if the RCD stated whether any effects (hyperplasia or neoplasia) were observed in the thyroids of treated F344 rats in these studies. Much higher doses were used in the F344 rat studies than in the Sprague-Dawley rat studies. Based on reductions in body weight gain observed in the F344 rat studies in the mid- and high-dose groups (approximately 20 and 40 percent, respectively), it appears that the doses used may have exceeded the maximum tolerated dose. It would be helpful to have information on survival, as well as other toxicity parameters to validate this assumption, and rule out the possibility that the presence of TBZ in the diet reduced palatability of the food, resulting in a reduction in food consumption.

5. Issues related to tumor findings, from the Proposition 65 perspective. Treatment related increases were reported for benign tumors at three sites (thyroid, preputial, and clitoral glands) in studies in rats. No treatment related increases in malignant tumors were reported. Under Proposition 65, this evidence in experimental animals, in and of itself, likely would be insufficient to identify TBZ as "clearly shown to cause cancer." However, it is possible that this evidence, taken together with positive genotoxicity data and with strong structural and functional analogies with known carcinogens, might be determined to meet the clearly shown criteria of Proposition 65. The lack of information presented in the draft RCD on structurally similar compounds, does not permit one to hazard a guess as to the likelihood of TBZ being listed as a carcinogen under Proposition 65.

Mutagenicity

I . We recommend expanding the discussion of mutagenicity of TBZ to address its potential for causing aneugenic effects (aneuploidy) in experimental systems (Mailhes et al., 1997; Aardema et al., 1998). Aneuploidy is the most prevalent of the various classes of human genetic disorders. It plays a significant role in adverse human health conditions including birth defects, pregnancy wastage, and cancer. Although at present there are insufficient data to determine with certainty if chemically induced aneuploidy contributes to human disease, it is prudent to address the aneugenic potential of chemicals in the human risk assessment process.

2. With regard to evaluating results in the Salmonella reverse mutation assay, it is important to point out that merely counting the number of positive versus negative studies fails to incorporate other potentially useful information, such as possible differences in study protocol (i.e., plate incorporation versus liquid suspension) and range of doses tested. The draft RCD appears to dismiss the positive findings reported by researchers at the National Institute of Environmental Health Sciences (NIEHS) and the National Toxicology Program (NTP) in Zeiger et al. (1988). Although it is true that Zeiger et al. present only summary findings, rather than raw data, the criteria used to classify findings as positive (i.e., reproducibility and apparent dose-response) are stated in the report. This positive finding of mutagenicity, as reported by the NIEHS/NTP, can not be dismissed, as it was reproducible and exhibited a dose-response.

3. On page 18 the draft RCD concludes that the genotoxic effects of TBZ are consistent with mitotic spindle dysfunction, caused by an inhibition of microtubule assembly. This is true for aneuploidy and polyploidy, and may be true for micronucleus induction, but would not explain the induction of SCEs, which presumably involves breakage and rejoining of chromatids. There are at least two published articles that report TBZ induces SCEs (de Pargament et al., 1987; Ardito et al., 1996). In addition, TBZ administered orally to mice tested positive for the induction of DNA breakage in the comet assay (Sasaki et al., 1997). Therefore, its clastogenic potential should not be discounted.

Developmental Toxicity

The effects seen from treatment on gestational day nine are substantially different than those seen from exposure on gestational days 7 to 15 or 6 to 15. In particular, Ogata et al. (1984) in experiment number three (gestational day nine) observed fusion of vertebral arches in 34/255 fetuses at 670 mg/kg. Fusion of vertebral arches was observed at other dose levels with appreciable frequency down to 240 mg/kg, and sporadically at 60 mg/kg. In contrast, Ogata et al. (1984) in experiment number one (gestational days 7 to 15) found 1/3 82 fetuses with fusion of vertebral arches at 700 mg/kg-day. Nakatsuka et al. (1995b) (gestational days 6 to 15) found no fusion of vertebral arches at-200 mg/kg-day. Thus, for this endpoint, the dose-response is very different for one day of exposure (gestational day nine) when compared to exposure for nine or ten days (gestational days 7 to 15 or 6 to 15). These differences in the data should be discussed and explained in the RCD, as well as the impact on the assessment of human risk.

Reproductive Toxicity

The section on reproductive toxicology appears to be complete and adequate for the purposes of the RCD. See "Specific Comments" below for additional comments. In the rat, reduced pup weight was observed with a NOAEL of 30 mg/kg-day. In the mouse, a NOAEL of 150 mg/kg-day was identified for reduced mice/litter and reduced pup weight.

Children as a Sensitive Subpopulation

I . The draft RCD concludes that children are more sensitive to TBZ exposure than adults. This conclusion is based on a wide spectrum of developmental toxicity in three species of laboratory animals (mouse, rat, rabbit) ranging from the induction of major malformations to fetal resorption (abortion). The lowest NOAEL identified for this type of developmental toxicity is 24 mg/kg-day based on fetal resorption and hydrocephaly in rabbits (first paragraph of page 26, page 43). The draft RCD suggests that the U.S. Environmental Protection Agency should consider an additional uncertainty factor to protect children in setting tolerances (page 43) even though the draft RCD does not include one in the assessment of dietary risk. This is an important issue and we feel that the incorporation of an additional uncertainty factor in the RCD to protect children and infants requires further discussion and consideration.

2. Additionally, it appears that the fetus is also particularly sensitive to the adverse effects of TBZ, based on the types of developmental toxicity observed in animal studies (i.e., skeletal abnormalities, fetal resorption, and hydrocephaly). Thus, it is suggested that women of child-bearing age also be considered a sensitive subpopulation.

Pharmacokinetics

This part of the draft RCD (pages 7 and 8) provides some information on the metabolism of TBZ after oral exposure to sheep, cow, goat, pig, human, and rat. It would be useful to include in the discussion any data on the toxicity of TBZ metabolites, and their role in the overall toxicological response from exposure to the parent compound. This section should also address the potential of TBZ, if any, to be distributed into milk.

Tolerance assessment

I . The draft RCD provides no information on which contaminated crops could lead to human exposure (as well as the levels of TBZ contamination), including processed foods such as orange juice, orange peel and marmalade. Such information is necessary for the risk characterization and should be included in the revised RCD.

2. No information is provided in the draft RCD on the types of TBZ residues considered for tolerance assessment (pages 44 to 46). We recommend that a more specific description of the residues (e.g., parent chemical only, parent chemical and its metabolites) be included.

3. We recommend providing a table with the estimates of the theoretical maximum residue contribution (calculated by using the tolerance level and a 100 percent crop treated assumption), the anticipated residue concentrations, and their representations as percentages of the acceptable daily intake, reference dose (RfD) or NOAEL for chronic exposure.

4. The tolerance assessment presented in the draft RCD qualitatively addresses the apparent greater sensitivity of children to TBZ exposure as compared to adults, but it does not quantitatively evaluate it. We recommend that the draft RCD include more discussion of whether the margin of exposure (MOE) calculations sufficiently account for the greater susceptibility of children. Current tolerances, and tolerances that take into account the greater sensitivity of children to TBZ should be presented for comparison.

Guidance and Advisories

I . Guidance and advisories such as a maximum contaminant level, permissible exposure limit, health advisory, reference exposure level or threshold limit value are not available for TBZ. The only available guidance for TBZ is the World Health Organization's (WHO) RfD of 0.3 mg/kg-day (page 4, second paragraph). The draft RCD includes the WHO RfD for TBZ but did not clearly state that there are currently no other guidance or advisories established for this chemical.

2. It would be useful to compare the chronic NOAEL(s) identified in the RCD with

the WHO RfD, discussing the toxicological bases for each one.

Environmental fate

I . Some information was provided in the draft RCD on the hydrolysis, photolysis, aerobic and anaerobic soil metabolism, and soil mobility of TBZ (page 5 and 6), but there are no data on TBZ behavior in the atmosphere. We recommend including a description of any data that pertain to atmospheric transport of TBZ, or to include a statement that the data are not available (or are not needed, in the event that only post-harvest application is anticipated).

2. We recommend including a conclusion regarding the persistence of TBZ in particular environmental media (e.g., soil, water, and air). Our independent review of the literature indicates that the expected mobility of TBZ in soil is low to slight and the half-life is 400 days (Wauchope et al., 1991). If released into water, TBZ will be essentially non-volatile (Wauchope et al., 199 1). If released into the atmosphere, TBZ will exist primarily in the particulate phase. In the vapor phase, it will degrade in the atmosphere by reaction with photochemically produced hydroxyl radicals with an estimated half-life of six hours (Meylan and Howard, 1993).

3. We recommend including a more complete characterization of TBZ in relation to the environment. Adverse ecological effects should be addressed. If this is outside the scope of the RCD, then a statement to this effect should be included.

Risk characterization

The "Risk Characterization" section of the draft RCD for TBZ (page 42) refers only to uncertainties in the current methodology and general assumptions used in the risk assessment. We recommend that this section include a discussion of the uncertainties specifically related to TBZ. These include the quality of the existing database, quality and limitations of the exposure data, data gaps (if any), and uncertainties related to toxicological responses, such as immunotoxicity and neurotoxicity for which risk assessment methodology have not been developed. The section on "Risk

Characterization" should also include a discussion on sensitive populations, including the unborn fetus, children and infants, and women of child-bearing age. Populations at special risk include people with any compromised liver functions.

Conclusions

According to the conclusions of the draft RCD, MOEs for potential acute and chronic exposures to workers handling and applying TBZ, and to the general public exposed via dietary consumption, were considered health protective. However, according

to the draft RCD, the current tolerance for TBZ in apples does not provide adequate protection from the toxic effects of this chemical for certain population subgroups. Most of the currently existing tolerances may not be health protective for children since they do not take into account their greater sensitivity to this chemical as compared with adults.

SPECIFIC COMMENTS

Page 7, first paragraph. "Thus, humans appeared to absorb at least 87% of a single oral dose of TBZ." It is not clear how this statement follows from the discussion before it.

Page 9, Table 1. Some of the categories should be IV rather than III.

Page 11, first paragraph. When it is stated that the liver and thyroid effects were reversible after 13 weeks, it could also be mentioned that this result supports a non-genotoxic mode of action of TBZ.

Page 11, first paragraph. The clearance rate of iodine was significantly elevated at the highest dose level only.

Page 11, Table 2. What are the units for the metabolic clearance rate of iodine?

Page 15, Table 5. For "kidney-tubular and/or ductal hyperplasia" in males, is 7/30 significantly different from 1/29 in the Fischer's exact test?

Page 19, first paragraph. Reproductive Toxicity/Summary. "The reproductive NOEL for mice was approximately 150 mg/kg-day, based on reduced numbers of mice formed and weaned per litter, as well as reduced weanling weight." The term "mice formed" is unclear. Does this refer to live litter size at birth, or something else? It would be helpful to use a more exact term. This change should be carried forward to the document summary on page 2.

Page 20, first paragraph. Reproductive Toxicity/Oral-Rat. "At 90 mg/kg-day, there was a significant ($P < 0.05$) decrement in pup weight gain. The NOEL for reduced pup weights was 30 mg/kg/day." It would be useful to specify when the decrement in pup weight was detected, and magnitude of decrement.

Page 20, third paragraph. Reproductive toxicity/Oral-mouse. "Mice (25/sex/group) were dosed with thiabendazole (purity unstated) at concentrations of 0, 0.02, 0. 1, or 0.5% in the diet for five generations (Nessel, 1981b). The NOEL was approximately 150 mg/kg-day for reduced numbers of mice formed and weaned per litter." Include an explanation

of how the dosage of 150 mg/kg-day was derived from the concentrations in feed. In addition, the term "mice formed" is unclear. It would be helpful to use a more exact term.

Page 20, fourth paragraph. Developmental Toxicity/Summary. "The Estimated No Effect Level (ENEL) for mice was 26 mg/kg-day, based on skeletal abnormalities." The data referred to are for a single administration of TBZ on gestational day nine (from Ogata et al. 1984, experiment number three). The endpoint and duration of exposure are different from the other mouse teratology study cited (Nakatsuka 1995b), so it is important to specify both endpoint and duration of exposure. In addition, perhaps use of the unit mg/kg should be used to denote a single exposure. The maternal and developmental NOAELs (25 mg/kg-day) and endpoints (maternal weight gain and fetal weight) for administration during gestational days 6 to 15 in mice (Nakatsuka 1995b) should be added to the summary. These changes should be carried forward to the document summary on page 2.

Page 20, fourth paragraph. Developmental Toxicity/Summary. "The NOEL for developmental toxicity in rabbits was 24 mg/kg-day based on fetal resorption and hydrocephaly. The material NOEL in rabbits was 120 mg/kg-day based on decrement in food consumption and body weight gain." There may be some question about the reliability of the developmental NOAEL from the first rabbit developmental toxicity study. The NOAEL from the second rabbit study (150 mg/kg-day) might be more reliable. Any change in the selection of the NOAEL should be carried forward to the document summary on page 2.

Pages 20, fourth paragraph. Developmental Toxicity/Summary. "In the rat gavage study, the NOEL for decreased maternal food consumption was 10 mg/kg-day." It appears that this NOAEL is for decreased maternal weight gain as well as food consumption.

Page 20, fifth paragraph. Developmental Toxicity/Gavage-mouse. "In the second regime, a single dosage of 2400 mg/kg-day was used on any one of days 6-12 of gestation. The third dosage regime was to give one of 17 dosages of thiabendazole, ranging from 30 to 2400 mg/kg-day, on day 9 of gestation." The units mg/kg-day usually refer to multiple exposures, for a single exposure the units mg/kg is more appropriate.

Page 20, fifth paragraph. Developmental Toxicity/Gavage-mouse. "The estimated no effect level (ENEL) (EDO I determined by probit analysis by the authors) for the incidence of 9 litters with fetuses having skeletal fusion was 26 mg/kg-day." The citation of "9 litters" appears to be an interpretation of Figure I from Ogata et al. (1984), which shows "probit" analysis, The "9" appears to come from the number of open circles associated with the line for limb deformities. However, closer inspection of this figure indicates that each data point represents all the litters at a given dose. For example, the solid black dot at about 2,400 mg/kg represents about 65 percent of all the litters at that dose having fetuses with skeletal fusion. This corresponds with the entry for 2,400 mg/kg in Table 7. There were 18 litters examined at 2,400 mg/kg, and a range of 18 to 28 litters at other doses. According to Table 7, there are 20 doses in the range of 30 to 2,400 mg/kg. Not all are apparent on Figure I because some data points coincide, and others are difficult to see because they fall on the X axis. The total litters analyzed would be about 20 x 20, or

400 litters. Deletion of "9" from the cited sentence would avoid confusion.

Pages 20 and 21, fifth paragraph. Developmental Toxicity/Gavage-mouse. "In a separate experiment, maternal weight loss was induced by starvation to see if weight loss alone would cause the appearance of skeletal malformations...." The term "starvation- is misleading. The more correct term is pair-feeding. It should be specifically pointed out in this paragraph that mice were pair-fed to thiabendazole-treated mice.

Page 21, second paragraph. Developmental Toxicity/Gavage-mouse "There were decrements in maternal body weight at 200 (15.6%), 400 (30.2%), and 800 mg/kg-day (39%), as well as reduced RBC counts, hemoglobin, and hematocrit." This sentence could be further clarified by noting that the numbers in parenthesis actually refer to the decrement in maternal body weight gain during treatment (i.e., on days 6 to 15 of gestation). There were also small decrements in maternal weight gain during treatment at 25 and 100 mg/kg-day, although these were not statistically significant. If the decrements at 25 and 100 mg/kg-day are not mentioned, then the qualifier "statistically significant" should be added to the above sentence.

Page 21, third paragraph. Developmental Toxicity/Gavage-mouse "There were slight decrements in body weight gain of the pregnant females at the high and middle doses in the first four days of the study." This paragraph omits the important observation that there were statistically significant decrements in maternal body weight gain during treatment in the 100 mg/kg-day (15 percent) and 200 mg/kg-day (24 percent) doses.
This should be added.

Page 21, third paragraph. Developmental Toxicity/Gavage-mouse "Differences in litter sizes, which were not due to treatment effects, caused the decrement in body weight gain to be more pronounced over the course of the study." The study cited (Nakatsuka 1995b) does not report extra-gestational weight gains. Thus, in the absence of further analysis, the above-cited sentence appears speculative and could be misleading in regard to the impact of litter sizes on body weight gains. It could well be that the change of extra-gestational weight gain was greater than of gestational weight gain.
Consideration should be given to omitting the phrase "caused the decrement in body weight gain to be more pronounced over the course of the study."

Page 21, paragraph 4. Developmental Toxicity/Gavage-rabbit "The NOEL for developmental toxicity was 24 mg/kg-day based on fetal resorption (4/18 litters resorbed with whole litter resorption at 120 mg/kg-day) and hydrocephaly (2 fetuses in 2 litters at 600 mg/kg-day and one fetus at 120 mg/kg-day)." It appears that the data cited in this study (Hoberman, 1989) are the same data as the "first" rabbit developmental study cited in the published report Lankas and Wise (1993). Based on these data, interpretation of the whole litter fetal resorptions observed at 120 mg/kg-day appears problematic. First, there were no whole litter fetal resorptions at the high dose in this study (600 mg/kg-day). Although it could be hypothesized that the abortions observed at 600 mg/kg-day were etiologically related, it is not clear that this is the case. Also, even if one assumes that they

are related, there would be little dose-response effect. At 120 mg/kg-day, there were 4/18 whole litter resorptions (no abortions), and at 600 mg/kg-day there were 4/13 abortions (no whole litter resorptions). Thus, increasing the dose by five times had little effect on the frequency of the whole litter resorptions plus abortions. While this is possible, it appears to leave a question about the biological meaning of the effects at 120 mg/kg-day. Second, in a subsequent study (cited in the draft RCD as Lankas and Wise 1991, apparently the same data as the "second" rabbit developmental study in Lankas and Wise 1993), which was nearly identical to the first study, there were no whole litter resorptions at 150 or 600 mg/kg-day. Finally, the frequency of whole litter resorptions at 120 mg/kg-day in the first study was not statistically significant ($p = 0.066$ by Fisher Exact test). Some of the same concerns apply to the observations of hydrocephaly. The small number of observations of the effect is far from statistically significant, either on a per litter or per fetus basis. Also, there were no observations of hydrocephalus in the second rabbit study at either 150 or 600 mg/kg-day. Overall, the results from the first study are difficult to interpret. Although there are indications of adverse developmental effects at the middle dose (120 mg/kg-day), various factors, including lack of statistical significance, lack of dose response, and lack of corresponding effects in the second study make interpretation difficult. The NOAELs from the second study appear to be more reliable for use in risk assessment.

Page 22, paragraph 2. Developmental Toxicity/Gavage-rat "A slight decrement in body weight gain (1.7 to 4.8%) was seen in the high dosage rats (80 mg/kg-day) on day s 8 through 14. Food consumption was significantly ($P<0.05$) reduced at dosages of 40 (11-15%) or 80 mg/kg-day (22-28%) compared to controls. The NOEL for decreased maternal food consumption was 10 mg/kg-day." In addition to the study which is cited in the draft RCD as Wise 1990, there is a published report, Lankas and Wise (1993), which appears to present either the same or a very similar study. In the latter report, it is stated that "There were statistically significant ($P = 0.05$) decreases in average maternal body weight gain in the 40 and 80 mg/kg/day groups between GD 6 and 18 (12% and 26% below control, respectively ...)." It would be helpful to reconcile the Lankas and Wise (1993) report with Wise (1990) article, particularly since the former report would indicate that the NOAEL should be for reduced food consumption and reduced maternal body weight gain.

Pages 22 to 24, Developmental Toxicity/Diet-rat. The results of the two rat dietary studies are difficult to reconcile. Both were conducted with Wistar rats, the first for gestational days 6 to 17 and the second for gestational days 7 to 17, but with very different concentrations of TBZ in the feed (up to 100 ppm in the first, and up to 10,000 ppm in the second). The results of the first study indicated reduced fetal body weights at 50 ppm but the results of the second study indicated no reduction at 1,250 and 2,500 ppm, but only at 5,000 and 10,000 ppm. Furthermore, the results first study indicated increased major skeletal malformations (including cleft palate) at 100 ppm, but the results of the second study did not indicate any skeletal malformations. Given that these effects are characteristic of a fairly developed embryo or fetus, it does not seem likely that these differences would result from one study starting on gestational day six and the other on gestational day seven. For these reasons, it may be appropriate to reconsider whether the results from the first (low concentration) study should be carried forward to the summary.

Page 22, last paragraph. The number of animals used per dose level is not indicated.

Page 24, Table 11. For skull bone hypoplasia, is 15 significantly different than 0?

Page 25, last paragraph. Three clinical studies are cited in which TBZ was administered acutely to treat parasitic infections. Is this a sufficient number, considering the large number of studies in the literature? In some of these studies the drug was administered for longer periods of time.

Page 25, last paragraph. "The NOEL in a third clinical study involving 42 male subjects was 3.3 mg/kg." What toxicity endpoint was this "NOEL" based upon?

Page 26, third paragraph. Since platelet counts were not elevated in the mouse, does this suggest that the thrombi were due to other factors?

Page 27, top paragraph. "through disruption of tubulin formation" should read "through disruption of microtubule formation."

Page 35, Table 16. For some categories the numbers in the third column do not equal the sum of the first and second columns.

Page 40, last paragraph. "but the increased clearance of iodinated compounds indicated an increased turnover rate for T3 and T4." This was only true at the highest dose level, not at the second highest dose level, where the effects of the test article on liver and thyroid weights and on TSH levels were also observed.

Page 41, second paragraph. "All other genotoxic effects involved spindle disruption ..." This is not the case for SCE induction, which presumably involves breakage and rejoining of chromatids.

Page 41, third paragraph, first sentence. It could also be added that according to Casarett and Doull's Toxicology (fifth edition, pages 617 to 62 1), the induction of thyroid adenomas by elevated TSH, resulting from liver stimulation, does not occur in humans.

Appendix, page 30, Table 2. The total dermal exposures for the citrus workers are not the sums of the hands, arms and T-shirt values.

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