Office of Environmental Health Hazard Assessment

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November 30, 2011

Greg W. Gorder, Ph.D.
Technology Sciences Group, Inc.
712 Fifth Street, Suite A
Davis, California 95616

Dear Dr. Gorder:

The Office of Environmental Health Hazard Assessment (OEHHA) has completed the review of your comments of December 21, 2010, concerning the possible listing of tetraconazole as causing cancer under the authoritative bodies provisions of Proposition 65.[1] We considered your comments in the context of the "Authoritative Bodies" listing regulations (Title 27, California Code of Regulations, section 25306). Our specific responses to your comments are provided in the enclosed document.

After a careful review of your comments, OEHHA has determined that tetraconazole meets the Proposition 65 criteria for listing as a carcinogen via the authoritative bodies mechanism. The listing is based on its formal identification by the U.S. Environmental Protection Agency, an authoritative body under Proposition 65. The next step in the listing process is the issuance of a notice of intent to list the chemical, which includes a 30-day public comment period.

Thank you for providing your comments and for your interest and participation in this process. Should you have any questions or need further assistance, please contact me at (916) 322-6325 or Martha Sandy at (510) 622-3190.

Sincerely,

Allan Hirsch
Chief Deputy Director

Enclosure

[1] The Safe Drinking Water and Toxic Enforcement Act of 1986, codified at Health and Safety Code, section 25249.5 *et seq.*

RESPONSE TO COMMENTS FROM TECHNOLOGY SCIENCES GROUP, INC. PERTAINING TO THE REQUEST FOR RELEVANT INFORMATION CONCERNING CONSIDERATION OF TETRACONAZOLE FOR LISTING UNDER PROPOSITION 65

Office of Environmental Health Hazard Assessment
November 2011

On October 22, 2010, the Office of Environmental Health Hazard Assessment (OEHHA) issued a request for relevant information concerning the possible addition of tetraconazole to the Proposition 65 list of chemicals known to cause cancer. The consideration of tetraconazole for listing is based on the U.S. Environmental Protection Agency's (U.S. EPA) identification of tetraconazole as causing cancer.

In a letter dated December 21, 2010, comments responsive to the request were provided by Dr. Greg W. Gorder of Technology Sciences Group, Inc. on behalf of Isagro USA, Inc. The submission included a comment letter as well as several research papers. Specific comments from Technology Sciences Group, Inc. are summarized in the numbered paragraphs below. OEHHA's response immediately follows each comment.

1. *In its determination on the carcinogenicity of tetraconazole in 2000, the U.S. EPA did not have all of the relevant scientifically valid data and documentation. This information would disqualify tetraconazole from further consideration under the authoritative bodies mechanism. This new data includes studies on the mechanism of mouse liver tumors for tetraconazole and structurally-related compounds and for phenobarbital and the relevance of these tumors to humans.*

   a. *The U.S. EPA evaluation did not consider the studies by Harada (1997) and Okasaki (1998) on tetraconazole. In the Harada studies, CD-1 BR mice treated for one or two weeks with tetraconazole showed induced microsomal protein and cytochrome P450 content of liver, and/or pentoxyresorufin O-dealkylase activity. Phenobarbital induced similar changes. In the Okasaki studies, CD Sprague Dawley rats treated for four or seven days with tetraconazole or phenobarbital showed increased liver mitotic index and proliferating cell nuclear antigen (PCNA)-positive cells at three days, with little change by seven days after treatment.*

   These unpublished studies were conducted before the 2000 U.S. EPA review of tetraconazole, although neither was cited in the review. As noted in the comment, the U.S. EPA did, however, describe other studies in mice and rats comparing the induction of liver enzymes by tetraconazole and phenobarbital in four-week studies in CD-1 BR mice and four-week studies in CD BR rats. The 1997 Harada studies in mice are in the same strain as the studies U.S. EPA considered in its 2000 review, and the study design and enzyme activity endpoints measured are very similar. The 1998 Okasaki studies in rats provide information on short-term effects on cell proliferation in the liver. However, the species tested was rats, and tetraconazole does not induce liver tumors in the rat. Thus, the 1998 Okasaki studies in rats are not informative with respect to the mechanism of mouse liver tumor induction by tetraconazole.

The comment does not describe why the additional unpublished information by Harada and Okasaki leads to a different conclusion regarding the carcinogenicity of tetraconazole, since U.S. EPA has noted some similarity to phenobarbital in terms of liver effects. These additional studies regarding liver effects support evidence already described by U.S. EPA in its determination and do not constitute substantial evidence that the regulatory criteria[1] have not been met.

b. Conazole compounds such as tetraconazole induce the same enzymes that are thought to trigger the same mechanism that causes phenobarbital-induced hepatocarcinogenicity. IARC has listed phenobarbital as "possibly carcinogenic to humans," but concluded there is "inadequate evidence" for its carcinogenicity in humans.

While some researchers have suggested that phenobarbital does not pose a carcinogenic risk to humans, there is no scientific consensus on this hypothesis. The U.S. EPA has not formally evaluated the carcinogenicity of phenobarbital. The International Agency for Research on Cancer (IARC) concluded that the evidence of carcinogenicity in humans is inadequate, namely, that "the available studies are of insufficient quality, consistency or statistical power to permit a conclusion regarding the presence or absence of a causal association between exposure and cancer, or that no data on cancer in humans are available." IARC did not conclude that there was human evidence suggesting a lack of carcinogenicity. Further, IARC did conclude that the experimental animal evidence is sufficient (IARC, 2001), and found that phenobarbital is possibly carcinogenic to humans (Group 2B). Importantly, IARC did not conclude that the mode of carcinogenic action was not relevant to humans.

The comment cites a review of rodent liver carcinogenicity by Holsapple *et al.* (2006). This review describes the IARC monograph and the more recent epidemiological study by Laminpää *et al.* (2002) as providing evidence that phenobarbital does not produce a carcinogenic effect in humans. However, as noted above, IARC concluded that the evidence in humans was inadequate to make such a conclusion. The investigation by Laminpää *et al.* examined cancer incidence among epileptic patients taking any of several antiepileptic drugs (carbamazepine, phenytoin, phenobarbital). Patients were followed-up at a mean of nearly 17 years. This study found statistically significant excesses of cancers of the brain and nervous system, larynx, liver, pancreas, colon, stomach, and lung. While some fraction of these tumors can be attributed to other causes, the authors concluded that "[t]he excess of some cancers might be attributable to enzyme-inducing antiepileptic drugs." Further, the study is not able to distinguish between the different specific antiepileptic drugs. Thus, this study does not provide evidence that phenobarbital does not cause cancer in humans.

The comment regarding conazoles relates to a hypothesized mechanism for chemically-induced liver carcinogenicity in rodents. The comment cites the Holsapple *et al.* (2006) review and published research papers by Juberg *et al.* (2006), Allen *et al.* (2006), Goetz *et al.* (2006), Ward *et al.* (2006), and Nesnow *et al.* (2009). Under the hypothesis referenced in the comment, the liver tumors in rats and

[1] Title 27, California Code of Regulations section 25306.

mice are not relevant to humans based on assumptions that both (1) tetraconazole causes cancer by the same mode of action as phenobarbital (and other conazole fungicides) and (2) the observed hepatocarcinogenicity of phenobarbital in rodents is not relevant to humans.

The hypothesized mode of action for phenobarbital discussed in the comment involves several key steps, including induction of CYP2B enzymes (in the P450 family) by activation of the constitutive androstane receptor (CAR), hepatocellular hypertrophy, hepatocellular proliferation, inhibition of programmed cell death (apoptosis), altered hepatocellular foci, and tumor development (Holsapple *et al.*, 2006). Holsapple *et al.* concede that important data gaps are "the extent to which this [phenobarbital-like mode of action] extends to other rodent hepatocarcinogens that are P450 inducers, the concordance of key events between rodents and humans, and a more detailed definition of the key events in rodents (*e.g.*, role of CYP2B, reactive oxygen species, DNA-methylation)." This review did not address tetraconazole specifically, so it does not provide any evidence regarding similarities in mode of action between tetraconazole and phenobarbital.

There is some evidence that triazole conazole fungicides have effects similar to phenobarbital. Studies have shown that several triazole conazole fungicides (other than tetraconazole) have exhibited some of the "key effects" in the hypothesized phenobarbital carcinogenic mode of action including activation of the CAR receptor, induction of CYP2B, hepatocellular hypertrophy and proliferation (Allen *et al.*, 2006). Studies identified in the comment, such as Juberg *et al.* (2006) also show liver enzyme effects of fenbuconazole similar to phenobarbital. Other studies have examined mice treated with different triazoles (other than tetraconazole) and found liver gene expression to vary suggesting possible differences in mechanism for liver toxicity (Goetz *et al.*, 2006; Ward *et al.*, 2006). Further, as noted by Allen *et al.* (2006), while several conazole triazole fungicides shared effects on mouse liver CYP enzymes and pathology, not all cause liver tumors in mice. This led these authors to conclude that other factors must be relevant to the potential carcinogenicity of these compounds. Nesnow et al. (2009) looked at transcriptional profiles for animals treated with phenobarbital, triadimefon, and propiconazole and found them to be significantly different. This led Nesnow *et al.* (2009) to conclude that the mechanisms of action for propaconazole and triadimefon are likely to differ. Overall, these studies do not present convincing evidence that the mechanism by which tetraconazole (or other conazole compounds) causes cancer is known, or known to be the same as phenobarbital. Therefore, these studies do not provide substantial evidence that the criteria of Section 25306 have been met.

2. *The determination that structurally-related conazoles also caused mouse liver tumors was important to the U.S. EPA's decision on tetraconazole. U.S. EPA has not identified any of the structurally-related conazoles it discusses as "likely to be carcinogenic to humans."*

   U.S. EPA's classification of tetraconazole as "likely to be carcinogenic to humans" was based on three weight-of-the evidence considerations. These included the strong liver tumor response observed in male and female mice, the relevance of these tumors to human exposures, as well as the hepatocarcinogenicity of

compounds structurally-related to tetraconazole. The 2000 U.S. EPA report is the most current assessment by the Agency that classifies tetraconazole's carcinogenicity, although the U.S. EPA has recently reiterated its classification (U.S. EPA, 2011). In its 2000 review of tetraconazole, U.S. EPA discussed nine structurally related conazole compounds, only one of which was classified as a B2 (probable) carcinogen (cyproconazole).[2] U.S. EPA considered the data on liver tumors across these different compounds to be relevant to its conclusion for tetraconazole. At least two other conazole compounds cause liver tumors in experimental animals and were classified by U.S. EPA as "likely to be carcinogenic to humans," imazalil in 1999 and epoxiconazole in 2001. Both of these conazole fungicides and phenobarbital are listed as known to cause cancer under Proposition 65.

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[2] Cyproconazole was re-evaluated in 2007, and classified as "not likely to be carcinogenic to humans at doses that do not cause a mitogenic response in the liver."

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