A WEIGHT OF EVIDENCE ASSESSMENT OF THE CARCINOGENIC POTENTIAL OF MANCOZEB
(CAS Number 8018-01-7)

PROPOSED NON-CLASSIFICATION AND NON-LABELLING BASED ON REGULATION (EC) No. 1272/2008 (CLP REGULATION), ANNEX VI, PART 2.

The carcinogenic potential of Mancozeb has been investigated in five chronic oncogenicity studies involving two species (rats and mice). The studies were designed based on approved guidelines and were conducted in accordance with the Principles of Good Laboratory Practices.

A review of the data reveals that chronic dietary feeding with Mancozeb may lead to the formation of tumors. However, the neoplasms are noted in only one species (rat), affecting only one organ (thyroid) at concentrations that are associated with systemic toxicity. Notwithstanding the findings of thyroid tumors in rats, using a weight of evidence approach, it is this reviewer's opinion that classification and labelling of Mancozeb based on Regulation (EC) No. 1272/2008 (CLP Regulation), Annex VI, Part 2 is not warranted due to:

- Mancozeb has a low carcinogenic potency
- Thyroid tumors are sex and species specific due to intraspecies variabilities.
- Thyroid tumors in rodents are biologically irrelevant to humans due to interspecies variabilities.

1. Evidence supporting the low carcinogenic potency of Mancozeb

    - ✓ Rodent studies indicate an interplay between genetic and non-genetic events in the development of thyroid tumors. The initiation step of carcinogenesis seems linked to genetic events with the induction of DNA mutations whereas the promotion step includes at least non-genetic events. A critical element in the evaluation of thyroid carcinogen is a determination of whether mutagenicity may account for the observed tumors. It can be said with assurance that the thyroid tumor found in Mancozeb rodent studies is not genetically derived. In fact,

Mancozeb was not genotoxic or clastogenic in any of the *in vitro* and *in vivo* mammalian and bacterial assays investigating all endpoints of mutagenicity - gene and point mutation, chromosomal damage and aneugenicity, and DNA damage and unscheduled DNA synthesis - in both the presence and absence of metabolic activation. The lack of genotoxicity is further demonstrated by negative mutagenic effects in *in vitro* photogenotoxicity study in the presence of UV light.

- ✓ The lack of a genotoxic mode of action suggests that Mancozeb has no intrinsic carcinogenic property and is not an initiator in carcinogenesis.
- ✓ Thyroid follicular cell tumors found in rats from chronic exposure to Mancozeb must then result from non-genetic events. A review of Mancozeb bioassays in rats reveals perturbations of thyroid and pituitary hormone status (decreased circulating thyroid hormone T3 and T4 levels and increased level of thyroid stimulating hormone [TSH]) in conjunction with thyroid follicular cell hyperplasia. These findings substantiate thyroid tumors found in rats were consequential to hormonal changes.
- ✓ Perturbation of the hypothalamus-pituitary-thyroid (HPT) axis leading to persistent stimulation of the thyroid gland by high level of TSH is a well-known mode of action for thyroid tumor formation.
- ✓ Mancozeb *per se* does not affect the HPT axis. However, it could be said with certainty that ethylene thiourea (ETU) – a main metabolite of Mancozeb – is directly involved. ETU is known to inhibit the activity of thyroid peroxidase, preventing iodide oxidation leading to decreased levels of circulating thyroid hormones T3 and T4 and increased level of TSH. Chronic perturbation of the HPT axis by ETU would result in morphological changes in the thyroid gland.
- ✓ Thyroid tumors are not unique to Mancozeb and actually are quite common in rats. In fact, spontaneous incidence for thyroid gland C-cell neoplasms in F344 rats occurs at a rate of 14.5% in males and 13.5% in females (Haseman et al., 1998).
- ✓ Most chemical carcinogens appear to be mutagenic and cause tumors in more than one site. Mancozeb is not mutagenic and produces tumor in only one site of one species.

- ✓ Collectively, the aforementioned facts support Mancozeb low carcinogenic potency.

2. Evidence supporting that thyroid tumor is sex-specific and species-specific due to intraspecies variabilities:

    - ✓ Thyroid follicular cell adenomas and carcinomas found in Mancozeb bioassays were noted only in Sprague Dawley rat at a dose level (750 ppm) that was associated with systemic toxicity (decreased body weight; presence of thyroid cell hyperplasia; and perturbations in blood thyroid hormone levels). No thyroid tumors were noted in rats (Crl: CD SD strain) fed Mancozeb up to 454 ppm for two years (1992).
    - ✓ In oncogenicity studies in mice (1991), no thyroid tumors were found even at dose higher than that in the rat bioassay (1000 ppm vs. 750 ppm). The absence of thyroid tumor development in mice may reflect the difference in metabolism of Mancozeb to ETU among species, confirming that quantitative differences in thyroid tumor formation exist across species.
    - ✓ The thyroid tumor incidences apparently were more pronounced in males than females. In the combined chronic/carcinogenicity study in rats (1990), the incidences of carcinomas were 23% in males and 7% in females. The incidences of thyroid adenomas were 33% in males and 10% in females. Similar observations were noted in multi-generation reproduction studies in rats (1988). At 1100 ppm, the incidences of follicular adenoma found in parental male and female rats were, respectively, 5/25 vs.1/25 of the F0 generation and 11/25 vs. 0/25 of the F1 generation. In another reproduction study, the incidences of follicular adenoma at 1200 ppm in parental male and female rats were, respectively, 3/25 vs. 0/25 of the F0 generation and 4/24 vs. 0/25 of the F1 generation.
    - ✓ Thyroid tumors were more prevalent in males than females since adult male rats have higher serum TSH levels than females (Chen HJ, 1984). The higher level of TSH associated with higher incidence of thyroid tumors in males substantiates the fact that TSH plays a significant role in stimulating DNA synthesis in cell proliferation leading to neoplasms.

3. Evidence supporting the lack of biological relevance of thyroid tumor in rodents to humans due to interspecies variabilities:

- ✓ The only known human thyroid carcinogen is ionizing radiation. It is not known with certain whether chemicals that affect thyroid cell growth lead to human thyroid cancer.
- ✓ Clinically manifest thyroid cancer in humans is uncommon and no chemical has been positively identified as being carcinogenic to the human thyroid.
- ✓ There are quantitative differences between humans and rodents about the thyroid physiology, the biological response of thyroid gland to TSH stimulation, the role of liver metabolism, etc.,
- ✓ The difference in interspecies sensitivity to TSH stimulation may be related to the presence of a high affinity binding protein, i.e., thyroxine binding globulin (TBG), which binds T4. TBG is found in humans and primates but is missing in rodents and lower vertebrates. Without TBG, T4 circulates more freely and is readily biotransformed and excreted. That explains the difference in T4 half-life between rats and humans. The half-life of T4 in rats is much shorter (< 1 day) than in humans (5-9 days). This difference in T4 half-life results in a 10-fold greater requirement for endogenous T4 in the rat thyroid than in the adult human. In rats, the constant demand in T4 would result in higher level of TSH in rats. Thus, the rodent thyroid gland is chronically stimulated by TSH levels to compensate for the increased turnover of thyroid hormone. Indeed, rodent TSH levels are probably 6 to 60-fold higher than in humans (Hill et al., 1998).
- ✓ Differences in rate of metabolism also contribute to the interspecies variabilities since the carcinogenic potential of Mancozeb is related to the formation of the metabolite ETU.
- ✓ Noteworthy to mention that epidemiology studies of humans exposed to occupational and environmental concentrations of Mancozeb do not show evidence of disruption of thyroid hormonal system and presence of thyroid tumors.
- ✓ Collectively, the aforementioned facts demonstrate that thyroid tumors in rodents are biologically irrelevant to humans.

Respectfully submitted,

Quang Q. Bui, PhD, DABT
Independent Toxicologist
25 Carriage Ln.,
North Tustin, California 92705, USA
Telephone: +1 714 559 2618
Email: quangqbuiphd@gmail.com

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