UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION PESTICIDES AND
TOXIC SUBSTANCES

November 30, 2000

MEMORANDUM

SUBJECT: ***Imazalil***: Response to the Registrant's Comments on the Human Health Risk Assessments and Science Chapter Reviews.

DP Barcode D270787 **PC code 111901**
Submission No.: S582653 **Case No. 2325**

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The Health Effects Division (HED) acknowledges the comments received from Janssen Pharmaceutica, Inc. on behalf of Janssen Pharmaceutica , Inc. and Makhteshim-Agan of North America, Inc (07/12/2000) on the toxicology and human health risk assessments of Imazalil.

The registrant disagrees with the HED toxicology assessment of this chemical in two areas:

- Lack of neurotoxicity of imazalil and consequently the inappropriate use of the 3X and 10X FQPA safety factors in the acute- and chronic-dietary risk assessments, respectively.
- Evidence of a threshold for liver tumor induction justifying the use of non-linear approach for Cancer Risk Assessment.

Registrant's Comment on Neurotoxicity Requirements: Janssen requested a waiver from the requirement for an acute neurotoxicity study based on the following:

- lack of evidence of neurotoxicity among the large number of imazlil studies
- the primary regulatory purpose for acute neurotoxicity studies is related to the endpoint for acute dietary risk. The current acute dietary endpoint has been derived from a developmental study in rabbits (most sensitive species) with a maternal and developmental NOAEL of 5 mg/kg/day in comparison to a maternal NOAEL in rats (the required species for acute neurotoxicity testing) that exceeded 40 mg/kg/day (*actually it was less than 40 mg/kg/day, the lowest dose tested).*
- the test doses required for an acute neurotoxicity study would be at least 3 to 10 fold greater than the current acute dietary endpoint.
- these concerns raise a question regarding what purpose an acute neurotoxicity study would serve in the risk assessment process. It is Janssen's experience that the Agency would continue to use the lower NOAEL from the rabbit developmental study for acute dietary hazard.

Janssen also stated that subchronic and developmental neurotoxiciy studies are not relevant to acute dietary risk and lack of these studies should not be applicable to assessing additional FQPA safety factors for acute dietary risk.

Janssen disagrees with the Agency's application of a 10X FQPA safety factor for chronic dietary risk and requests the Agency to lower this safety factor for the following reasons:

- lack of increased susceptibility of rat, rabbit and mouse fetuses to *in uteri* exposure in developmental studies.
- no evidence of enhanced susceptibility in pups when compared to adults in in the pre/post natal two generation reproduction study in rats.
- no evidence of abnormalities in the development of the fetal nervous system in the pre/post natal studies.
- no concern for positive neurological effects from the available neurotoxicity and other toxicology studies. Janssen questioned the results of Tanaka 1995 study because of lack of positive control or historical control data and further stated that it was not a standard developmental neurotoxicity study in rats.

HED's Response: The lack of evidence of neurotoxicity in the various imazalil studies was recognized by the HIARC and FQPA committees. However, the positive findings in the Tanaka mouse study cannot be dismissed in spite of the lack of method validation as well as measurements of parameters examined in a standard developmental neurotoxicity study. HIARC also determined that a postnatal developmental neurotoxicity study in rats with imazlail is required based on alterations in the nervous system in developmental studies with structurally related compounds. In the developmental toxicity studies with tebuconazole, there was evidence

2

of alterations to the development of the fetal nervous system in mice (increased malformations of the brain and spinal cord and exencephaly), in rats (anophthalmia) and in rabbits (neural tubule defects characterized as meningocoele and spina bifida and hydrocephalus) at doses 10 fold higher than the developmental toxicity NOAEL. With the exception of Etaconazole and Hexaconazole, other structurally related compounds (Triadimefon, Triademenol, Bitertanol, Uniconazole, Propiconazole, Azaconazole, and Cyproconazole) have been shown to cause developmental toxicity at levels below those that are maternally toxic. For these reasons, an acute, subchronic and developmental nerurotoxicity studies are required. Consequently the FQPA safety factors of 3X and 10X are required for acute and chronic dietary risk assessments, respectively.

Registrant's Comments on Cancer Risk Assessment: Janssen disagrees with the Agency's classification of imazalil in the category "likely to be carcinogenic in humans". Janssen believes that the available studies clearly demonstrate that induction of liver tumors in both rats and mice is a threshold effect and that the use of the multistage linearalized model should be reconsidered. The liver is the primary target organ of imazalil toxicity in many subchronic, chronic and multigeneration studies. Clear and consistent NOAEL's and LOAEL's were seen for the liver effects. The tumorigenic responses in male rats and male mice were only seen at high doses producing significant microsomal enzyme induction and liver changes consistent with enzyme induction (increased liver weight, hypertrophy and vacuolation). No tumorigenic responses were seen in female mice or rats. Liver and thyroid tumors have not been observed at doses that do not induce microsomal enzyme and associated liver pathology. Janssen recently submitted a mechanistic study report (MRID 45160101) evaluating the interrelationship between hepatic microsomal enzyme induction and thyroid tumorignesis in male rats. Treatment related effects on the levels of thyroxine (T4) and thyroid stimulating hormone (TSH) were apparent. Additionally. Imazalil was nonmutagenic *in vivo* and *in vitro* and showed cell proliferation in the liver at a very high oral dose and at cytotoxic levels in an *in vitro* and *in vivo* unscheduled synthesis (UDS) which was negative for genetic toxicity. Taken collectively, these results demonstrate a threshold for both liver effects and for tumorigenic effects.

HED's Response: The CARC classified imazalil in the category "Likely to be carcinogenic in humans" based on increases in hepatocellular adenomas and combined liver adenomas/carcinomas in **male** Swiss albino mice and Wistar rats (CARC Final report dated December 7, 1999; HED Doc # 013885). In male rats, there was also an increased incidence of combined thyroid follicular cell adenomas/carcinomas. Imazalil was **non mutagenic** in *in vitro* and *in vivo* mutagenicity assays. Imazalil is structurally related to triazole compounds, which are hepatocarcinogens in mice. The Committee recommended a linear low-dose (Q_1*) extrapolation approach for the quantification of human cancer risk based on the most potent liver tumors in mice. This approach was supported by the lack of confirmation of the mode of action for the induction of liver tumors. In the published literature, Imazalil was found to induce glutathione S-transferase positive (GST-P) foci in rat liver (Hasegawa and Ito, 1992). The **CARC concluded** that the available data were inadequate to establish the role of imazalil as a liver tumor promoter. The Registrant recently submitted a mechanistic study (MRID 45160101)

3

for thyroid tumor induction in male rats. The HED reviewers noted that the T4 and TSH levels were generally correlated with each other in mid- and high-dose rats and support the hypothesis that imazalil alters thyroid hormone homeostasis in male rats resulting in hypothyroidism leading to thyroid tumor induction. Although the mode of action for thyroid tumor induction in male rats supports the non-linear approach for cancer risk assessment, the Agency still maintains its position regarding the linear-low dose extrapolation approach because the liver tumors in mice were induced at lower dose than in rats. This approach is supported by the lack of compelling evidence for the mode of action for liver tumors in mice.

In addition, the registrant noted some errors in the Toxicology Chapter dated 9 February, 2000 (HED Doc. No. 013993). These along with the Agency's response are presented below:

Registrant's Comment (page 2): a statement should be added to the last sentence to clarify that pup survival rate from birth to post natal day 4 in the F_2 generation was significant only at the highest dose tested (HDT).

HED Response: The statement will be added to that effect in the revised toxicology chapter as well as in the risk assessment document.

Registrant's Comment (page 9): The registrant stated that in section 4.3, 2nd paragraphh, 5th line, the developmental toxicity LOAEL/NOAEL should be "40/10" rather than "120/80" as noted on the second paragraph on page 11.

HED Response: As reported in the DER of this study (MRID # 44578201) the developmental toxicity LOAEL and NOAEL are 120 and 80 mg/kg/day, respectively.

Registrant's Comment (page 35): The registrant stated that in the 2nd row of the table, the findings under the LOAEL: 400 ppm should include increased centrilobular swollen hepatocytes in males at 1 month and slightly swollen cortical cells in the adrenal of one 400 ppm and two 800 ppm female rats observed at the 3 month terminal sacrifice.

HED Response: The revised toxicology chapter will reflect the change requested.

Registrant's Comment (page 35): The use of the "symbol" for males and females in the Table as well as the terms "males" and "females" is not needed.

HED Response: The revised revised toxicology chapter will reflect the change requested.

Registrant's Comment (page 36): The table (5th row, results column) indicates that the developmental NOAEL for MRID 44567802 was 10 mg/kg/day (not 40) and the LOAEL was 40 mg/kg/day based on increased resorption and decreased litter size.

HED Response: The revised toxicology chapter will reflect the change requested.

4

Registrant's Comment (page 41): The word "male" on line 5 in the table should be inserted between "in" and "Wistar" to denote the sex specificity of the thyroid adenomas and carcinomas to this sex.

HED Response: The revised toxicology chapter will reflect the change requested.

5

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6