# IMAZALIL (addendum)

*First draft prepared by*
*U. Mueller[1] and Roland Solecki[2]*

*[1]Office of Chemical Safety ,Therapeutic Goods Administration, Canberra ACT, Australia; and*
*[2] Safety of Substances and Preparations—Coordination and Overall Development,*
*Federal Institute for Risk Assessment, Berlin, Germany*



## Explanation

Imazalil (synonym: enilconazole, a pharmaceutical), 1-[2-(2,4-dichlorophenyl)-2-(2-propenyloxy)ethyl]-1*H*-imidazole), was first evaluated by the JMPR in 1977 when a temporary acceptable daily intake (ADI) of 0–0.01 mg/kg bw was established. The ADI of 0–0.01 mg/kg bw was reaffirmed in 1986 on the basis of the no-observed-adverse-effect level (NOAEL) in a 2-year study in dogs. In 1991, the JMPR reconsidered imazalil and a new ADI of 0–0.03 mg/kg bw was established based on a NOAEL for clinical signs, decreased body-weight gain and food consumption, decreased serum concentration of calcium, increased alkaline phosphatase activity, and increased liver weight in a study in dogs. In 2000, the JMPR reaffirmed the ADI and concluded that an acute reference dose (ARfD) was unnecessary.

The present Meeting was asked by the Codex Committee on Pesticide Residues to reconsider the need for an ARfD in view of refinements to the criteria used to establish ARfD values since 2000.

The present Meeting considered six new studies of acute toxicity (investigating end-points such as death and irritation) that were submitted by the sponsors. The Meeting also reconsidered the existing database on imazalil as previously evaluated and described in the monograph; however, the original studies were not available to the Meeting.

All the studies submitted complied with the essential elements of the applicable test guidelines and GLP requirements.

## Evaluation for acute reference dose

### 1. Toxicological studies

#### *1.1 Acute toxicity*

The results of studies on the acute toxicity of imazalil in rats and rabbits are summarized in Table 1.

*Rats*

Groups of five male and five female fasted Sprague-Dawley rats were given a single dose of imazalil technical (purity not reported) at 400, 438, 480, or 679 mg/kg bw by gavage in PEG 400. The animals were observed daily for mortality and clinical signs for up to 14 days after dosing. Body weight was recorded on days 0, 7, 14 or at death. All rats found dead and those that survived the observation period underwent gross pathological examination. No rats at doses of less than 679 mg/kg bw died. Deaths (four out of five for each sex) at 679 mg/kg bw occurred 1 or 2 days after dosing. Body-weight gain among survivors was similar. Clinical signs of toxicity observed within 1–4 h after dosing included lethargy, hunched posture, piloerection, ptosis and and lowered respiration rate. In both sexes these clinical signs were observed in all groups on day

***Table 1. Acute toxicity of imazalil***

| Species | Strain | Sex | Route | LD50 (95% CI or range) (mg/kg bw) | Purity (%) | Reference |
|---|---|---|---|---|---|---|
| Rat | Wistar | Male | Oral | 343 (262–448) | > 95 (nitrate) | Niemegeers (1979) |
| | | Female | | 288 (221–377) | | |
| | | Male | | 343 (262–448) | 100.4 (technical) | Niemegeers (1979) |
| | | Female | | 227 (174–297) | | |
| | | Male | | 355 (272–464) | 102.7 (sulfate) | Niemegeers (1979) |
| | | Female | | 309 (237–404) | | |
| | | Male | | 371 (284–485) | 99.1 (acetate) | Niemegeers (1979) |
| | | Female | | 309 (237–404) | | |
| Rat | SD | Male | | 664 (595–742) | NS (technical) | Dreher (1990a) |
| | | Female | | 664 (595–742) | | |
| Rabbit | NZW | Males and females | Dermal | > 2000 | 97.6 (technical) | Teuns et al. (1990a) |
| Rat | SD | Males and females | | > 2000 | NS (technical) | Dreher (1990b) |
| Rat | Wistar | Male | Inhalation[a] | > 2000 mg/m$^3$ | 99.5 (technical) | Appelman & Woutersen (1983) |
| | | Female | | > 2000 mg/m$^3$ | | |
| Rat | SD | Male | Inhalation (dust, 33%; < 4 μm, nose-only) | 2880 mg/m$^3$ | NS (technical) | Blagden (1990) |
| | | Female | | 1840 mg/m$^3$ | | |

CI, confidence interval; NS, not stated; NZW, New Zealand White; SD, Sprague-Dawley

[a] Deployed as smoke from a smoke generator.

1, but were no longer evident by day 2 at 400, 438 and 480 mg/kg bw. The clinical signs persisted until day 6 in the male survivor and day 5 for the female. The gross pathological examinations of animals that died revealed haemorrhagic lungs and dark or patchy pallor livers. No abnormal findings were observed among the remainder at the terminal sacrifice on day 14 (Dreher, 1990a).

Groups of 10 male and 10 female fasted Wistar rats were given imazalil base (technical) or one of three of its salts, namely nitrate, acetate or sulfate (purities were 100.4%, > 95%, 99.1%, and 102.7% respectively) as a single dose at 160, 320, or 640 mg/kg bw by gavage. The animals were observed daily for mortality and clinical signs for up to 14 days after dosing. No deaths occurred at 160 mg/kg bw and all rats died at 640 mg/kg bw. Most deaths at 640 mg/bw died on day 1 of dosing and all were dead by day 2. Clinical signs of toxicity that were observed in both sexes at 160 mg/kg bw on day 1 included ataxia, piloerection, hypotonia, hypothermia, and ptosis. Tremors, lacrimation and salivation were restricted to females at 160 mg/kg bw. At higher doses, all clinical signs observed at 160 mg/kg bw occurred in both sexes (Niemegeers, 1979).

Groups of five male and five female Sprague-Dawley rats received imazalil technical (purity not reported) as a single dose at 2000 mg/kg bw, moistened to a paste with water, applied to an area of 10 $cm^2$ of shaved intact skin. After semi-occlusion of the application site for 24 h, the test substance was removed and the skin was washed with warm water and dried. The animals were observed for 4 h after dosing and then daily for mortality and clinical signs. Body weights were recorded on study days 0, 7 and 14. At the end of the 14-day recovery period, all rats underwent gross pathological examination. No rats died and no clinical signs of toxicity were observed during the study. No dermal irritation was observed and no gross lesions were found at necropsy (Dreher,1990b).

Groups of five male and five female Sprague-Dawley rats received a single nose-only exposure to imazalil (purity not reported) at a concentration of 1970, 3150 or 4570 mg/$m^3$ (1.97, 3.15 or 4.57 mg/l air) for 4 h in a chamber. The mean mass median aerodynamic diameter of the dust in the chamber ranged from 5.1 to 6.2 μm, with 39.6% of the particles at 5.1 μm and the remainder at 6.2 μm. The animals were observed daily for mortality and clinical signs for 14 days after dosing, and were weighed daily. All rats found dead and those which survived the observation period underwent gross pathological examination. No rats survived exposure at 4570 mg/$m^3$, but two out of five males survived at 3150 mg/$m^3$. All males and three out of five females survived exposure at 1970 mg/$m^3$. Clinical signs of toxicity in survivors after exposure were lethargy, hunched posture, piloerection, ataxia and lowered respiration rate. Clinical signs gradually regressed so that by day 6–14 the rats appeared normal. There were no differences in body-weight gain among survivors. Necropsy in decedents revealed dark lungs, patchy pallor of the liver, and haemorrhage of the small intestine. No abnormal findings were present in survivors (Blagden, 1990).

Groups of five male and five female Wistar rats were exposed to technical imazalil (purity, 99.5%) at concentrations ranging between 6300 and 20 670 mg/$m^3$ (6.3 and 20.67 mg/l air) for 4 h in a chamber. The concentration of imazalil in the chamber was generated by burning a candle containing 5 g of imazalil and measured at intervals during the 4-h exposure. The diameter of all airborne particles was reported to be < 7 μm. During the first 30 min of exposure, none of the animals could be observed owing to the density of the smoke and for the remaining duration of the study, in less dense smoke, the rats' eyes were partially closed. No rats died during exposure or the subsequent 14-day observation period. There was some loss in body weight during the first 2 days after exposure. There were no gross pathological changes observed at necroscopy on day 14 (Appelman & Woutersen, 1983).

*Rabbits*

Groups of five male and five female New Zealand White rabbits were given imazalil technical (purity, 97.6%) as a single dose at 2000 mg/kg bw, moistened to a paste with water, and applied to a shaved intact measuring about 10% of body surface area under occlusion for 24 h. The test substance was removed and the skin was washed with warm water and dried. The animals were observed daily for mortality and clinical signs. Body weights were recorded on study days 0, 7 and 14. At the end of the 14-day recovery period, all rabbits underwent gross pathological examination. No rabbits died. The only clinical sign was sedation on day 1, but this was no longer evident by day 2. No dermal irritation was observed and no gross lesions were found at necropsy (Teuns et al., 1990a).

The potential of imazalil to cause primary irritation of the skin was studied in three New Zealand White rabbits (two males, one female) that received a single application of technical-grade imazalil (purity not stated) at a dose of 0.5 g to 6.25 $cm^2$ of skin for 4 h under a semi-occlusive dressing. The animals were observed for 1 h and then daily for 14 days, and irritation was scored according to the Draize method. Very slight erythema was observed at two out of three sites after 1 and 24 h, but then in only one out of three sites at 48 h. In a third rabbit erythema was slight after 1 h and then well defined by 24 h, and had then regressed to being slight by 48 h. Very slight oedema was observed 24 h at two out of three sites, but this had also regressed so that no sites were affected after 48 h. It was concluded that, under the conditions of the study, imazalil can be classified as being a mild skin irritant (Dreher, 1990c).

The potential of imazalil to cause primary irritation of the eye was studied in three New Zealand White rabbits (one male, two females) that received a single instillation of 0.1 ml of technical-grade imazalil (purity not stated) into the left conjunctival sac. The animals were observed for 21 days, and the eyes were scored according to the Kay & Calandra method. The material was moderately irritating (mean score, 33.7). All eyes appeared normal by day 7. It was concluded that, under the conditions of the study, imazalil can be classified as being a moderate eye irritant (Dreher, 1990d).

*Guinea-pigs*

The sensitization potential of technical-grade imazalil (purity not stated) was examined in 20 Dunkin-Hartley female guinea-pigs, which were induced by intradermal injection of 1% imazilil in arachis oil followed by epicutaneous challenge with 50% (w/w) powder in arachis oil. Groups of 10 controls given the solvent and vehicle were included. The animals were scored for sensitization 24 h and 48 h after challenge according to the Magnusson & Kligman scale. The sensitization rate was 0%. It was concluded that imazalil was not a sensitizer to guinea-pig skin (Dreher, 1990e).

### *1.2 Reproductive toxicity*

*(a) Multigeneration study*

In a two-generation study, groups of 24 male and 24 female Wistar rats were given diets containing imazalil (purity, 98.0%) at a nominal dose of 0, 5, 20, or 80 mg/kg bw per day for 60 days before mating (both sexes), during a 3-week mating period (both sexes), during gestation, and until weaning of the first generation. The $F_0$ males were killed after mating. The first generation ($F_1$) received imazalil during growth, mating, gestation, and weaning of the second generation ($F_2$). The $F_1$ males were killed after mating. The doses during the premating period were equal to 0, 4.2, 18, and 70 mg/kg bw per day for males and 0, 5.0, 22, and 100 mg/kg bw per day for females. During the $F_0$ gestation, the intakes were 0, 4.0, 16, and 87 mg/kg bw per day,

and those during the $F_1$ gestation were 0, 4.3, 19, and 88 mg/kg bw per day. Thus, the intakes tended to be slightly lower than the nominal doses. The animals were observed daily for general health, appearance, and behaviour. Body weights were recorded weekly before mating and, for females, on days 1 and 22 after copulation. Body weights were also recorded during the 3-week lactation period. Food consumption was recorded weekly before mating for both sexes and during gestation and lactation for females. The body weights of $F_1$ and $F_2$ pups in each litter were recorded 8–12 h after parturition and on days 4, 14, and 21. The kidneys, liver, and reproductive organs of the parental and $F_1$ generations were examined histologically.

Two $F_1$ females at the highest dose were killed in extremis, and one $F_2$ female at the highest dose and one in the control group died soon after parturition or during lactation. $F_0$ males at the highest dose showed decreased weight gain before mating; a similar but less pronounced effect was seen in females before mating, but an effect was also seen in $F_1$ and $F_2$ dams during gestation, at parturition, and at days 4, 14, and 21 of lactation. No effects on weight gain were seen in other treated groups, and there was no clear effect on food consumption in any group. Histopathological changes (liver vacuoles) were seen in $F_0$ males at the highest dose, but no lesions were seen in the male reproductive system at any dose. Decreased numbers of corpora lutea were seen in $F_0$ females. At the highest dose, both $F_1$ and $F_2$ generations had considerably reduced numbers of live pups and larger numbers of stillborn pups, and the $F_1$ generation had fewer implantations; the duration of gestation was increased in both generations, by approximately 1 day, with an associated increase in the incidence of dystocia. The survival rates of pups during lactation were decreased in all $F_1$ treated groups on days 4, 14, and 21; in the $F_2$ generation, survival was decreased at all times at the highest dose and on days 14 and 21 at the lower dose; at the intermediate dose, the survival was comparable to that of controls, so that there was no dose–response relationship. No differences in body weight were seen between $F_1$ and $F_2$ pups. No teratogenic effect was observed.

The NOAEL for maternal toxicity was 20 mg/kg bw per day on the basis of reduced maternal weight gain. The NOAEL for fetotoxicity was 20 mg/kg bw per day on the basis of decreased numbers of live pups and increased numbers of stillbirths at 80 mg/kg bw per day. As summarized above, the survival rates of $F_1$ pups during lactation were decreased in all treated groups on days 4, 14, and 21 of lactation and in the $F_2$ generation at 5 mg/kg bw per day (days 14 and 21) and 80 mg/kg bw per day (days 4, 14, and 21); moreover, there was no dose–response relationship for the effect in the $F_1$ generation at day 4, although there was at days 14 and 21. It is arguable that the data, especially for days 14 and 21, indicate a dose-related effect, but there was clearly no dose-related effect in $F_2$ pups. There is no biologically plausible explanation for the difference between the two generations, and a statistical re-examination on a litter basis showed that the mortality rate was significantly increased only in the group at the highest dose. As hepatotoxicity was observed in $F_0$ males at the highest dose, the NOAEL for paternal toxicity was 20 mg/kg bw per day (Dirkx et al., 1992b; see also Janssen Pharmaceutica NV, 1994).

*(b) Developmental toxicity*

*Mice*

Twenty-four female Cobs mice received imazalil sulfate orally at a dose of 0, 2.5, 10, or 40 mg/kg bw per day from day 6 to day 16 of gestation. All animals were killed on day 19 of gestation. No effects were found on mortality, body weight, food consumption, pregnancy rate, number of live, dead, or resorbed fetuses, pup weight, or mean litter size. During gross observations, talipes valgus (club foot) was observed in all groups, the incidence not being significantly different at any dose when compared to the control group. No additional abnormalities were seen after radiographic examinations and Lapras' sectioning technique (Marsboom et al., 1985) (Annex 1, reference 62).

In a study of developmental toxicity, groups of 30 inseminated Cobs CD-1 mice were given imazalil (purity not stated) at a dose of 0, 40, 80, or 120 mg/kg bw per day by gavage on day 6 to

day 16 of gestation. Treatment did not affect the maternal mortality rate, but maternal body-weight gain and food consumption were decreased at the two higher doses. Litter size and the number of live pups were reduced in all treated groups. The number of resorptions was increased at the highest dose. No teratogenic effects were seen, and there were no differences between groups in mean pup weights.

The NOAEL for maternal toxicity was 40 mg/kg bw per day. A NOAEL for fetotoxicity could not be identified in view of the decreases in litter size and number of live pups at all doses (Gillardin et al., 1987) (Annex 1, reference 89).

In another study of developmental toxicity, groups of 30 inseminated Cobs CD-1 mice were given imazalil (purity, 99.3–99.5%) at a dose of 0, 10, 40, 80, or 120 mg/kg bw per day by gavage on days 6 to 16 of gestation. Deaths were recorded during the study, and the animals were examined daily for abnormal clinical signs. Body weights were determined on day 1, daily on days 6–17, and on day 19 after copulation. Food consumption was recorded over days 1–5, days 6–16, and days 17–18. On day 19 after copulation and before killing, all survivors underwent a complete physical examination. The surviving dams were then killed, and macroscopic changes were recorded; the weights of the uteri were recorded after removal of that organ in toto. The dams were examined for numbers of live and dead fetuses, implantation sites, and embryos undergoing resorption. All live fetuses were weighed, and live and dead fetuses were examined for external abnormalities. The pups were sexed and the fetuses examined radiographically. The fetuses were then dissected, and bones were stained with alizarin.

Fourteen mice died during the study: one control, four at 80 mg/kg bw per day, and nine at 120 mg/kg bw per day. At the highest dose, piloerection, excitability, convulsions, hypothermia, and prostration were observed. Body weights were decreased on days 17 and 19 in mice at the two higher doses in comparison with concurrent controls; moreover, the body-weight gain of these animals was decreased during days 6–17 after copulation, and that of animals at 40 and 120 mg/kg bw per day was decreased during days 17–19 after copulation. The total body-weight gain (uterine weight subtracted) over the whole period of 1–19 days was decreased at doses of 40 mg/kg bw per day and more. Food consumption was decreased during dosing on days 6–16 in mice at 80 and 120 mg/kg bw per day and after dosing in those at 40 mg/kg bw per day and more. The pregnancy rates and number of implantations were comparable among groups, whereas fewer live fetuses were found in animals at the highest dose, and the number of resorptions was increased. The body weight of pups was decreased at the highest dose, but the sex ratio was similar in all groups. The frequency of minor changes in ribs was increased at 40 and 80 mg/kg bw per day but not at the highest dose; there was thus no clear dose–response relationship. The number of major abnormalities was comparable in all groups.

The NOAEL for maternal toxicity was 10 mg/kg bw per day on the basis of decreased body-weight gain at 40 mg/kg bw per day and reduced food consumption after treatment. In addition, deaths occurred at doses of 80 mg/kg bw per day and above. The NOAEL for fetotoxicity was 80 mg/kg bw per day as the number of live fetuses was reduced, the number of resorptions was increased, and the body weights of the pups were decreased at the highest dose. There was no evidence of teratogenicity (Levron et al., 1991) **(**Annex 1, reference 89).

If the results of the two studies of developmental toxicity in mice are taken together, the overall NOAEL for maternal toxicity (reduced body-weight gain) and fetotoxicity (reduced number of live fetuses, bodyweight and an number of resorptions) was 10 mg/kg bw per day.

*Rats*

Groups of 20 female rats were fed imazalil nitrate at a dose of 0, 5, 20 or 80 mg/kg bw per day from day 6 to day 15 of gestation. Fetuses were delivered by caesarean section on day 21. There were no differences between the control group and the test groups in regard to average number of implantations, number of live or dead fetuses, distribution of embryos or resorbed

fetuses in either uterine horn, weights or number of pups, or body-weight gains, food consumption, survival, and number of pregnancies among the dams. No abnormalities were noted among the fetuses in the control and various test groups with waved ribs in one litter of the group at 80 mg/kg bw per day (Marsboom, 1970) (Annex 1, reference 28).

In a study of developmental toxicity, groups of 24 pregnant Sprague-Dawley OFA.SD (IOPS Caw) rats were given imazalil (sulfate; purity, 99.9%) at a dose of 0, 40, 80, or 120 mg/kg bw per day by gavage on days 6–16 of gestation. The dams were examined daily and weighed 1, 6, 17, and 22 days after copulation. Food consumption was recorded during days 1–5 (before dosing), 6–16 (during dosing), and 17–21 (after dosing). At the end of the study, on day 22 of gestation, the dams were killed and examined for live and dead fetuses, implantation sites, and resorptions. Live fetuses were weighed, and live and dead ones were examined for anomalies radiographically and by alizarin staining to visualize skeletal anomalies. One animal at 40 mg/kg bw per day died, but this death was considered to be unrelated to treatment as no others occurred during the study. Maternal body weight was reduced at the end of dosing at all three doses on day 17 and at the highest dose on day 22, in comparison with concurrent controls. Decreased body-weight gain was seen at the highest dose. Decreased food consumption was seen at all three doses throughout treatment. The rates of pregnancy and numbers of corpora lutea and implantations were similar in all groups. The number of live fetuses was decreased and the number of resorptions increased at 120 mg/kg bw per day. At the two higher doses, the birth weights of the pups were decreased. No teratogenic effects were seen. The NOAEL for fetal toxicity was 40 mg/kg bw per day, on the basis of reduced pup weight at the next higher dose. No NOAEL for maternal toxicity could be identified because of decreased maternal body weight at all doses (Gillardin et al., 1988) (Annex 1, reference 89).

*Rabbits*

Groups of 20 female New Zealand White rabbits received imazalil nitrate (purity, 99.2%) at a dose of 0, 0.63, or 2.5 mg/kg bw per day by gavage in water on days 6 to 18 of pregnancy. All surviving animals were killed on day 28 of pregnancy. Four females died during the test (three in the control group and one in the group at 0.63 mg/kg bw per day). Body-weight gain of the dams was decreased at both doses, as well as average litter size, the percentages of live fetuses, and 24-h survival rates. The percentages of resorbed fetuses increased with increasing dose. Retrospective statistical analysis showed that the effects on maternal body weight, litter size, and number of resorptions were not statistically significant. A small number of anomalies in all groups (hydrocephaly, fused ribs, and deformed legs) were considered to be non-dose related. Individual data were not available to the Meeting. In this study embryotoxic and maternally toxic effects were observed at both doses. Therefore, this study was repeated at lower dosages, as described in the next paragraph (Marsboom, 1974) (Annex 1, reference 89).

Groups of 15 female New Zealand White rabbits received imazalil nitrate (purity, 97.8%) at a dose of 0, 0.16, or 0.63 mg/kg bw per day by gavage in water from days 6 to 18 of pregnancy. The surviving animals were killed on day 28 of pregnancy. Mortality (three dams in the group at 0.16 mg/kg bw per day died during the study), body weight, and pregnancy rate were not significantly different among the groups. Mean litter size was normal in all groups, and no statistically significant differences were seen with regard to the number of live, dead, or resorbed fetuses, birth weight, or 24-h survival rate. Fetal skeletal examination and fetal sectioning revealed no compound-related abnormalities (Marsboom & Dirkx, 1981) (Annex 1, reference 89).

In a study of developmental toxicity, groups of 15 pregnant New Zealand White rabbits were given imazalil (purity, 99%) at a dose of 0, 1.2, 2.5, or 5 mg/kg bw per day by gavage on days 6–18 of gestation. The animals were weighed before the start of dosing, at day 19, and at termination. Food consumption was not recorded. On day 28 of gestation, the animals were killed

and examined for the numbers of live and dead fetuses, implantation sites, and resorptions. Live fetuses were weighed, and dead and live fetuses were examined for anomalies radiographically and by alizarin staining. One rabbit at the lowest dose and one at the highest dose died. There was no clear effect of treatment on body-weight gain or pregnancy rate. No differences were seen between groups in the numbers of live or dead fetuses or resorptions or in the birthweights of pups or 24-h survival rate. The NOAEL for both maternal and fetal toxicity was 5 mg/kg bw per day (Dirkx & Marsboom, 1985) (Annex 1, reference 89).

In another study of developmental toxicity, groups of four pregnant albino rabbits were given imazalil sulfate (purity, 98.2–100%) at a dose of 0, 5, 10, or 20 mg/kg bw per day by gavage on days 6–18 of gestation. The animals were observed daily for ill health, abnormal behaviour, or other clinical effects. Body weights were recorded on the morning of day 0 and on days 6, 19, and 28 of gestation. Food consumption was recorded during days 0–5, 6–19, and 19–28. On day 28 of gestation, the surviving animals were killed and autopsied, the uterus was removed and the weight recorded, and the dams were examined for the numbers of dead and live fetuses and resorptions. The numbers of corpora lutea were determined, and the pups were sexed. Live fetuses were weighed, and both live and dead fetuses were examined externally by radiographic examination and alizarin staining. The fetuses were then dissected.

Eight animals at 20 mg/kg bw per day and one at 10 mg/kg bw per day died during the experiment. Five of those at the highest dose that died showed weight loss. No significant differences in clinical end-points were found in the survivors. On day 19 of gestation, the body weight of animals at 20 mg/kg bw per day was decreased in comparison with that of concurrent controls. Food consumption was reduced in animals at 10 mg/kg bw per day in comparison with concurrent controls during days 0–5, and in animals at 10 and 20 mg/kg bw per day during days 6–18. The pregnancy rates and numbers of corpora lutea were comparable between groups. Animals at 20 mg/kg bw per day had a decreased number of live pups, and those at 10 and 20 mg/kg bw per day had increased numbers of resorptions. Although these changes were not statistically significant, the numbers were small, and the decrease may be biologically significant. There was a significant decrease in the number of male fetuses at 5 mg/kg bw per day, but not at higher doses. Fetuses of does at 20 mg/kg bw per day had an increased incidence of thirteenth rib and missing sternum in comparison with concurrent controls, but, especially in the latter case, there was no clear dose–response relationship, and the Meeting considered these findings to be coincidental.

The NOAEL for maternal toxicity was 5 mg/kg bw per day on the basis of reduced food consumption at 10 mg/kg bw per day. The NOAEL for fetal toxicity was also 5 mg/kg bw per day on the basis of an increased number of resorptions and a decreased number of live pups at 10 mg/kg bw per day (Dirkx et al., 1992a) (Annex 1, reference 89).

### *1.3 Special studies: developmental neurotoxicity*

A study of reproductive toxicity with measurement of neurobehavioural end-points was reported in which groups of 10 male and 10 female Crj: CD-1 mice were given diets containing imazalil (purity, > 99%) from age 5 weeks in the $F_0$ generation to age 9 weeks in the $F_1$ generation. The compound was given at a concentration of 0, 0.012, 0.024, or 0.048% (0, 120, 240, or 480 ppm, if percentage is assumed to equal w/w), resulting in intakes for the $F_0$ generation of 0, 19, 31, and 79 mg/kg bw per day for males and 0, 26, 45, and 100 mg/kg bw per day for females before conception; 0, 18, 34, and 74 mg/kg bw per day during mating; 0, 21, 38, and 83 mg/kg bw per day during gestation; and 0, 65, 150, and 260 mg/kg bw per day during lactation. The intakes in the $F_1$ generation were 0, 21, 41, and 87 mg/kg bw per day for males and 0, 24, 49, and 93 mg/kg bw per day for females.

In the $F_0$ generation, there was no effect on the body weight of either sex before conception or on the body weights of dams during gestation or lactation. Food intake was increased in

females at the lowest and highest doses before conception, but not in those at the intermediate dose. A number of indicators of exploratory behaviour were increased in males at the highest dose. In the $F_1$ generation, litter size and weight and sex ratio at birth were unaffected by treatment. The mean body weight of animals at the two higher doses was reduced in early lactation. Indicators of behavioural developmental were affected, as follows. In males, surface righting was affected at the two higher doses on postnatal day 4 and at the intermediate dose on postnatal day 7. Swimming head angle was affected in males at the highest dose at postnatal day 4. In females, surface righting was affected in all treated $F_1$ offspring on postnatal day 7, without a clear dose–response relationship, and swimming head angle was affected in females at the highest dose on postnatal day 4. No convincing dose–response relationships were seen for other observations, such as on multiple water T-maze performance at week 7. By week 8, there were no effects on the exploratory behaviour of either sex. The doses used in this study were higher than those in other studies reviewed in this monograph. Furthermore, although the results suggest that neurobehavioural end-points can be adversely affected in mice exposed to diets containing imazalil during development, comparison with the study of Levron et al. (1991), described above, suggests that the lowest dose might have been almost maternally toxic. In view of the inconsistent results at the lowest dose, the many end-points measured, and lack of dose–response relationships in the adverse outcomes observed, the NOAEL was the lowest dose tested, about 20 mg/kg bw per day (Tanaka, 1995).

## 2. Observations in humans

Imazalil was used at oral therapeutic doses of up to 1200 mg over 6 months for the treatment of chronic fungal infection with *Alternaria alternata* in a single female patient. Oral administration was initiated at a dose of 50 mg daily and gradually increased to 1200 mg daily. The drug was tolerated without evident toxicity based on haematology and clinical observations with the exception of the induction of nausea at high-dose levels. Measurements of blood levels found rapid clearance and no bioaccumulation even after repeated administration at high doses (Stiller & Stevens, 1986).

## Comments

*Toxicological data*

The present Meeting evaluated studies that gave the following results:

The acute oral $LD_{50}$ of imazalil technical and its salts in rats ranged between 200 and 664 mg/kg bw. Clinical signs that were observed at 160 mg/kg bw and above in survivors were ataxia, piloerection, hypotonia, hypothermia, and ptosis.

Imazalil was a slight irritant to the skin of rabbits and was moderately irritating to the eye. It had sensitizing potential when tested by the Magnusson & Kligman method.

The 2000 JMPR evaluated studies that gave the following findings:

In a series of studies of developmental toxicity in mice, rats and rabbits, the lowest NOAEL for maternal toxicity after dosing by gavage was 5 mg/kg per day on the basis of reduced food consumption at higher doses. No teratogenicity was seen in any species. The lowest NOAEL for fetal toxicity in rabbits was 5 mg/kg bw per day on the basis of an increased number of resorptions and a reduced number of live pups.

In a two-generation study, the NOAEL for maternal toxicity was 20 mg/kg bw per day on the basis of reduced body-weight gain. The NOAEL for fetotoxicity was 20 mg/kg bw per day on the basis of a decreased number of live pups and an increased number of stillbirths at 80 mg/kg bw per day. A statistical re-examination on a litter basis showed that the rate of mortality of $F_1$ pups during lactation was significantly increased at the highest dose of 80 mg/kg bw per day.

In two studies of developmental toxicity in mice, the overall NOAEL for maternal toxicity (reduced body-weight gain) and fetotoxicity (reduced number of live fetuses, body weight and number of resorptions) was 10 mg/kg bw per day.

A published case study involving only one woman indicated that imazalil used to treat a fungal infection was well tolerated after oral ingestion at high doses (50 mg per day progressing to 1200 mg per day over 6 months). The only adverse effect noted was nausea.

## Toxicological evaluation

The Meeting considered that an ARfD was necessary on the basis of mortality at doses of less than 1000 mg/kg bw, and acute clinical signs at and above 160 mg/kg bw.

On the basis of the data reviewed and previous evaluations, the Meeting established an ARfD of 0.05 mg/kg bw, using the NOAEL of 5 mg/kg bw per day for maternal and fetal toxicity in a study of developmental toxicity in rabbits and a safety factor of 100. It was considered that this ARfD would also be protective of the potential effects observed during gestation and lactation. The Meeting concluded that it would be inappropriate to use a human case study with only one individual for the purpose of establishing an ARfD.

The Meeting recognized that an ARfD based on maternal and fetal toxicity would be conservative for the general population, but that in the absence of a more suitable study this value was considered appropriate.

### *Levels relevant to risk assessment*

| Species | Study | Effect | NOAEL | LOAEL |
|---|---|---|---|---|
| Rat | Two-generation reproduction study[a] | Maternal toxicity | 20 mg/kg bw per day | 80 mg/kg bw per day |
| | | Fetotoxicity | 20 mg/kg bw per day | 80 mg/kg bw per day |
| Rabbit | Developmental[b] | Maternal toxicity | 5 mg/kg bw per day | 10 mg/kg bw per day |
| | | Developmental toxicity | 5 mg/kg bw per day | 10 mg/kg bw per day |

[a] Dietary administration at a nominal concentration

[b] Gavage administration

*Estimate of acute reference dose*

0.05 mg/kg bw

*Information that would be useful for the continued evaluation of the compound*

Additional end-points relevant for establishing an ARfD

## References

Appelman, L. & Woutersen, R. (1983) Acute inhalation toxicity study of an imazalil containing smoke, developed by a smoke-generator in rats. Unpublished report No. V83.308/230831 from Civo Institutes TNO, Zeist, Netherlands. Submitted to WHO by Janssen Pharmaceutica NV.

Blagden, S.M. (1990) Imazalil technical: acute inhalation toxicity study four-hour exposure (nose only) in the rat. Unpublished report No. 306/3 (MCW report No. R-5740) dated 23 November 1990 from Safepharm Laboratories Limited, Derby, UK.

Dirkx, P. & Marsboom, R. (1985) R 18531 oral embryotoxicity and teratogenicity study in New Zealand White rabbits (segment II) administration: oral. Unpublished report No. 1482 (85.18.04) from Department of Toxicology, Janssen Pharmaceutica NV, Beerse, Belgium. Submitted to WHO by Janssen Pharmaceutica NV. Conforms to OECD guideline for testing of chemicals: teratogenicity No. 414, 12 May 1981. GLP: 21CFR part 85.35 (b) (7), 58.35 (c).

Dirkx, P., Lampo, A., Coussement, W. & Van Cauteren, H. (1992a) R 23979—imazalil sulphate. Embryotoxicity and teratogenicity study in albino rabbits. Administration: orally by gavage. Unpublished report No. 2615 from Department of Toxicology, Janssen Research Foundation, Beerse, Belgium. Submitted to WHO by Janssen Pharmaceutica NV.

Dirkx, P., Lampo, A., Vandenberghe, J., Coussement, W. & Van Cauteren, H. (1992b) R 23979—imazalil 2-generation reproduction study with 1 litter per generation in Wistar rats. Administration orally through the diet. Unpublished report No 2337 from Department of Toxicology, Janssen Research Foundation, Beerse, Belgium. Submitted to WHO by Janssen Pharmaceutica NV.

Dreher, D.M. (1990a) Imazalil technical: acute oral toxicity test in the rat. Unpublished report No. 306/1 (MCW report No. R-5738) dated 24 July 1990 from Safepharm Laboratories Limited, Derby, UK.

Dreher, D.M. (1990b) Imazalil technical: acute dermal toxicity (limit test) in the rat. Unpublished report No. 306/2 (MCW report No. R-5739) dated 19 July 1990 from Safepharm Laboratories Limited, Derby, UK.

Dreher, D.M. (1990c) Imazalil technical: acute dermal irritation test in the rabbit. Unpublished report No. 306/4 (MCW report No. R-5741) dated 19July 1990 from Safepharm Laboratories Limited, Derby, UK.

Dreher, D.M. (1990d) Imazalil technical: acute eye irritation test in the rabbit. Unpublished report No. 306/5 (MCW report No. R-5742) dated 29 July 1990 from Safepharm Laboratories Limited, Derby, UK.

Dreher, D.M. (1990e) Imazalil technical: Magnusson & Kligman maximisation study in the guinea pig. Unpublished report No. 306/6 (MCW report No. R-5743) dated 19 July 1990 from Safepharm Laboratories Limited, Derby, UK.

Gillardin, J.M., Sanz, G. & Marsboom, R. (1987) R 27180 embryotoxicity and teratogenicity study in COBS CD1 mice (segment II): oral by gavage. Unpublished report No. 86-07 (86.09.18) from Research Department, Laboratoires Janssen, Aubervilliers, France. Submitted to WHO by Janssen Pharmaceutica NV.

Gillardin, J.M., van Cauteren, H., Sanz, G. & Marsboom, R. (1988) R 27180 embryotoxicity and teratogenicity study in Sprague-Dawley rats (segment II): oral by gavage. Unpublished report No. 2003/88-05 from Research Department, Laboratoires Janssen, Aubervilliers, France. Submitted to WHO by Janssen Pharmaceutica NV.

Janssen Pharmaceutica NV (1994) Amendment to report No. 2337 (Dirkx, P., Lampo, A., Vandenberghe, J., Coussement, W. & Van Cauteren, H. (1992) 2-Generation reproduction study with imazalil technical grade with 1 litter per generation in Wistar rats, when orally administered through the diet. Department of Toxicology, Janssen Research Foundation, Beerse, Belgium. Submitted to WHO by Janssen Pharmaceutica NV.

Levron, J.C., van Cauteren, H., Sanz, G. & Marsboom, R. (1991) Imazalil sulfate R 27180S embryotoxicity and teratogenicity study in COBS CD1 mice. Unpublished report No. 2374/90-06 from Département de Recherche, Laboratoires Janssen, Aubervilliers, France. Submitted to WHO by Janssen Pharmaceutica NV.

Marsboom, R. (1970) Potential of R18531 for embryotoxicity and teratogenic effects in rats receiving R18531 orally. Unpublished report from Janssen Pharmaceutical. Submitted to the WHO by Pennwalt Corporation.

Marsboom, R. & Dirkx, P. (1981) Imazalil: R 18531. Oral embryotoxicity and teratogenicity study in New Zealand White rabbits (Segment II). Unpublished experiment No. 10816 from Janssen Research Laboratories. Submitted to WHO by Janssen Pharmaceutica NV, Beerse, Belgium.

Marsboom, R. (1974) Potential of oral R 18531 for embryotoxicity and teratogenic effects in rabbits. Unpublished report No. 535 from Janssen Research Laboratories. Submitted to WHO by Janssen Pharmaceutica NV, Beerse, Belgium.

Marsboom, R., Gillardin, J.M., & Sanz, G. (1985) R 27180 Imazalil. Embryotoxicity and teratogenicity study in COBS mice. Unpublished experiment No. 85-02 from Janssen Research Laboratories. Submitted to WHO by Janssen Pharmaceutica NV, Beerse, Belgium.

Niemegeers, C. (1979) Comparative acute oral toxicity studies of the different salts of imazalil in rats. Unpublished report No. N17417 from Department of Pharmacology, Janssen Research Foundation, Turnhoutseweg, Beerse, Belgium. Submitted to WHO by Janssen Pharmaceutica NV.

Stiller, R.L., & Stevens, D.A. (1986) Studies with a plant fungicide, imazalil, with vapor-phase activity, in the therapy of human alternariosis, *Mycopathologia*, **93S**, 169–172.

Tanaka, T. (1995) Reproductive and neurobehavioral effects of imazalil administered to mice. *Reprod. Toxicol.*, **9**, 281–288.

Teuns, G., Ligtvoet, T., Coussement, W., Lampo, A., van Cauteren, H. & Marsboom, R. (1990a) Study of the acute dermal toxicity of imazalil: R 23979 (technical grade) in New Zealand White rabbits. Unpublished report No. 2344 from Department of Toxicology, Janssen Research Foundation, Turnhoutseweg, Beerse, Belgium. Submitted to WHO by Janssen Pharmaceutica NV.