

DIMETHOATE (addendum)

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Explanation

Dimethoate is an organophosphate ester, and virtually all its toxic effects are due to the inhibition of acetylcholinesterase activity. JMPR evaluated dimethoate for toxicological effects in 1963, 1965, 1967, 1984, 1987, and 1996. An acceptable daily intake (ADI) of 0–0.002 mg/kg bw was established in 1996 on the basis of the apparent NOAEL of 1.2 mg/kg bw per day for reproductive performance in a study of reproductive toxicity in rats and with a safety factor of 500. Although a safety factor of 100 would normally be used in deriving an ADI from a study of this type, the Meeting was concerned about the possibility that reproductive performance might have been affected at 1.2 mg/kg bw per day in this study and therefore used a higher-than-normal safety factor. No data were available to assess whether the effects on reproductive performance were secondary to the inhibition of cholinesterase activity. The 1996 JMPR concluded that it was not appropriate to base the ADI on the results of studies of volunteers, since the crucial end-point (reproductive performance) had not been assessed in humans. The present review was undertaken to consider the need for establishing an acute reference dose (RfD) and to evaluate new studies submitted by the sponsor.

Evaluation for acute reference dose

1. Neurotoxicity in rats

1.1 Acute neurotoxicity

(a) Administration by gavage

In a study that complied with the United States Environmental Protection Agency good laboratory practice (GLP) regulations, 40 CFR part 160, and guideline 81-8-SS, groups of 12 male and 12 female Sprague-Dawley Crl:CD BR rats (aged 43 days) were given dimethoate (purity, 99.1%) as a single dose at 0, 2 or 20 mg/kg bw by gavage in water. Additional groups of 15 male and 15 female rats received dimethoate at a dose of 200 mg/kg bw.

Observations and examinations were carried out until day 14 after dosing, when surviving animals were sacrificed. Viability, clinical signs and body weight were recorded. A functional observational battery (FOB) and motor activity evaluations were performed before treatment, at the time of peak effect, 2 h after dosing (as determined in a dose range-finding study), and on days 7 and 14. Brain weight and dimension were measured at termination. All animals were perfused in situ and neuropathological examinations were carried out on five randomly selected animals of each sex in the control group and in the group receiving a dose of 200 mg/kg bw.

No mortalities were observed during the study. A lower body-weight gain was measured in males over days 0–7 (–38%) at 200 mg/kg bw. The most remarkable clinical signs at this dose were observed on days 1 and/or 2 and included gait alterations (rocking, lurching or swaying), tremors (whole body or forelimbs/hindlimbs) and constricted pupils. Other clinical signs at 200 mg/kg bw consisted mainly of coloured material on the body and decreased defecation, and were observed on days 1, 2 and/or 3, but persisted until day 5 for one male. The most notable effects on FOB parameters in males and females were alterations in posture, convulsions, tremors and changes in faeces consistency during the home cage observations; lacrimation, salivation and changes in fur appearance during the handling observations; impaired mobility, gait alterations and decreased rearing activity during the open-field observations; alterations in approach, touch, startle, tail pinch and pupil responses, forelimb extension and air righting reflex during the sensory observations; reduced hindlimb extensor strength, reduced forelimb grip strength and impairments in rotarod performance during the neuromuscular observations; increased catalepsy times and decreased body temperature during the physiological observations at 200 mg/kg bw. Additionally, treatment-related reductions in mean ambulatory and total motor activity in males and females were apparent at this dose. The effects were noted approximately 2 h after treatment on day 0, and were transient in nature (gait alterations, tremors and constricted pupils persisted until days 1 and/or 2 for some animals), but on the basis of cage-side observations some symptoms persisted until day 5. None of the above signs were apparent on days 7 or 14. The only treatment-related effect on FOB parameters at 20 mg/kg bw was the absence of pupil response (5/12 males and 6/12 females affected versus 0/12 and 2/12 among the controls). No treatment-related changes in brain weights and dimensions or in central or peripheral nervous system tissues examined microscopically were observed at any dose. The presence of one female (of the five examined) at 200 mg/kg bw with minimally swollen sciatic nerve axons (none in concurrent control group) is not considered to be significant compared with the maximum incidence of 17% among historical controls.

The NOAEL for acute neurotoxicity in rats treated by gavage was 2 mg/kg bw on the basis of an absence of pupil response at ≥ 20 mg/kg bw (Lamb, 1993).

(b) *Administration in the diet*

Groups of 24 male and 24 female Sprague-Dawley Crl:CD BR VAF Plus rats (aged 8–9 weeks) received diet containing dimethoate (purity, 99.1%) providing a single nominal dose of 0, 1, 2, 3 or 15 mg/kg bw, in a study that complied with United States Environmental Protection Agency GLP regulations, 40 CFR part 160, and guideline 82-1. In a 2-week feeding adaptation phase before administration of the test material, the animals were conditioned to eat their daily ration of food over a short period of time. During this phase, animals received two-thirds of their daily food ration for 1 h between 06:00 and 07:00 and one-third of their daily ration of food for 1 h between 17:00 and 18:00. The test material was administered once on day 1 during the first daily feeding. Calculated intakes of dimethoate, which were close to the intended intakes, were 0, 0.96, 1.95, 2.94 and 15.01 mg/kg bw per day for males and 0, 0.98, 2.11, 3.23 and 15.01 mg/kg bw per day for females. For the remainder of the study, rats had access to control diet ad libitum until termination on day 15. Doses and time of peak effect (2.5–3 h after dosing) for FOB and/or detailed clinical observation, and for effects on cholinesterase activity were determined in two preliminary studies. Eight animals of each sex per dose were sacrificed for determination of cholinesterase activity (in plasma, erythrocytes and brain) on day 1, at time of peak effect. The remaining 16 animals of each sex per group were subjected to FOB and determination of locomotion activity on day 1 (time of peak effect) and on day 15. After these observations on day 15, all animals were sacrificed and determinations of cholinesterase activity were conducted on eight animals of each sex per group, and gross necropsy was conducted on the remaining eight animals of each sex per group. Clinical observations and determination of body weights and food consumption were made during the study. No histopathological examination was carried out. Determinations of cholinesterase activity (also measured before dosing in all animals) involved reaction with acetylthiocholine and 5,5-dithio-bis(2-nitrobenzoic acid) (DTNB). The separated plasma and erythrocyte samples and brain sections were stored frozen at -70°C for up to 2 weeks if determination of cholinesterase activity was not possible at the day of sample collection.

There were no deaths or treatment-related clinical signs of toxicity and no effects on body weight or food consumption. FOB parameters and examination of motor activity showed no effects that could be attributed to the test material. Plasma cholinesterase activity was statistically significantly reduced on day 1 at 15 mg/kg bw in males (50% of control value) and in females (60% of control value); no significant reduction was observed on day 15. Erythrocyte cholinesterase activity was statistically significantly reduced on day 1 at 15 mg/kg bw in males (42% of control value) and in females (35% of control value) and at 3 mg/kg bw in males (71% of control value), while the reduction in males at 2 mg/kg bw (75% of control value) was not statistically significant. No significant differences were observed on day 15. Brain cholinesterase activity was significantly reduced on day 1 in both sexes at 15 mg/kg bw (hippocampus: 67% and 62% of control value in males and females, respectively; cortex: 64% and 57% of control value in males and females, respectively; striatum: 59% and 53% of control value in males and females, respectively) and in females at 3 mg/kg bw (cortex, 89% of control value). On day 15, inhibition of cholinesterase activity in the hippocampus was still statistically significant in both sexes at 15 mg/kg bw (84% and 85% of control value in males and females, respectively). There were no treatment-related effects on weight of the brain or on cholinesterase activity in the three regions of the brain investigated, and no macroscopic findings on days 1 or 15.

The NOAEL was 2 mg/kgbw on the basis of a statistically significant inhibition of cholinesterase activity in erythrocytes in males (29%) and in the brain cortex in females (11%) at ≥ 3 mg/kgbw. Recovery at 14 days after dosing appears to be incomplete at the highest dose of 15 mg/kgbw, since there was still a slight but statistically significant reduction of cholinesterase activity (15–16%) in the hippocampus (Schaefer, 1999).

1.2 Short-term study of neurotoxicity

In a study that complied with EPA GLP regulations, 40 CFR part 160, and guideline 82-7, groups of 10 male and 10 female (12 male and 12 female at the highest dose) Sprague-Dawley strain Crl:CD BR rats (aged 50 days) were given diets containing dimethoate (purity, 99.1%) at a concentration of 0, 1, 50 or 125 mg/kg for 91, 92, 93 or 94 days. These dietary concentrations provided intakes of 0, 0.06, 3.22 or 8.13 mg/kgbw per day for males and 0, 0.08, 3.78 or 9.88 mg/kgbw per day for females. Rats were randomly assigned to one of four study replicates to conduct FOB and tests for locomotor activity. Five rats of each sex from the control group and from the groups receiving the lowest and intermediate doses, and seven males and five females from the group receiving the highest dose were allocated for determination of plasma, erythrocyte and brain cholinesterase activities; the remaining five rats of each sex per group were allocated for neuropathological investigations. Clinical observations were recorded daily, body weights and food consumption were measured weekly, FOB and locomotor activity were assessed at weeks 3, 7 and 12. Cholinesterase activity was measured in plasma and erythrocytes before dosing and at weeks 3, 7 and 13, and in brain at week 13. Animals were not fasted before blood collection. Cholinesterase measurements in brain, and initial measurements in blood were performed according to the method of Dietz et al. (1973); this method uses the Ellman reaction, but the substrate is propionylthiocholine, not acetylthiocholine as proposed by Ellman et al. (1961). Later in the study, measurements of cholinesterase activity in erythrocytes and plasma were conducted at another laboratory by a method that used the Ellman reaction with acetylthiocholine as the substrate. All samples were stored at -20°C until analysis. Erythrocytes, plasma and brain from week 13 were stored for 3–7 days until analysis. Brains were weighed and dissected for measurement of brain cholinesterase activity at termination. Tissue from the central and peripheral nervous system was dissected and processed for histopathological examination after perfusion.

No mortalities occurred during the study. Small faeces in females at 50 mg/kg and in males and females at 125 mg/kg were noted primarily during weeks 2–6 of treatment. At 125 mg/kg, the mean body weights of males were slightly reduced during weeks 3–13, and cumulative mean body-weight gains were decreased in weeks 0–1 to 0–13. No treatment-related effects on food consumption, FOB (home cage, handling, open field, sensorimotor, neuromuscular and physiological observations) and locomotor activity (total and ambulatory motor activity) were apparent at any dose. Plasma cholinesterase activity was statistically significantly inhibited in males at 50 mg/kg (weeks 7 and 13) and at 125 mg/kg (weeks 3, 7 and 13). Erythrocyte cholinesterase activity was statistically significantly inhibited at week 7 and 13 in both sexes at 50 mg/kg (53% and 51% of control value in males; 66% and 58% in females) and at 125 mg/kg (40% and 47% of control value in males; 45% and 43% in females). At 1 mg/kg, erythrocyte cholinesterase activity in males was slightly, but statistically significantly reduced at week 7 (89% of control value), but no significant inhibition was seen at week 13. Statistically significant inhibition of cholinesterase activity was observed at 125 mg/kg in both sexes in the following brain regions: olfactory region (82% of control value in males), midbrain (85% of control value in males; 82% in

females), brainstem (83% of control value in males; 80% in females) and cortex (88% of control value in males). No differences were seen in absolute or relative brain and brain-region weights and no treatment-related effects were observed on neuropathological examination.

The NOAEL for systemic toxicity and neurotoxicity was 1 mg/kg (equal to 0.06 mg/kg bw per day) on the basis of inhibition of erythrocyte cholinesterase activity (34–49%) and small faeces at ≥ 50 mg/kg (equal to ≥ 3.22 mg/kg bw per day) (Lamb, 1994).

1.3 Developmental neurotoxicity

Groups of 24 pregnant female Crl:CD BR rats (aged 10–11 weeks) were given dimethoate (purity, 99.1%) at a dose of 0, 0.1, 0.5 or 3 mg/kg bw per day by oral gavage in water from day 6 of gestation until postnatal day 10; offspring received the same doses by oral gavage on postnatal days 11–21. This study complied with United Kingdom GLP regulations 1999, European Commission Directive 1999/11/EC, OECD GLP principles 1997, and EPA guideline OPPTS 870.6300. Clinical observation was performed on all dams at least twice daily throughout the study. Body weight and food consumption were recorded several times throughout the study; parturition and duration of gestation were noted. Ten dams per group were subjected to a detailed clinical examination and open arena observations on days 12 and 18 of gestation, and on postnatal days 4 and 10. On postnatal day 4, litters were culled to eight pups each (four males and four females when possible). Five pups from each litter were allocated to undergo further functional investigations (motor activity on postnatal days 13, 17, 22 and 59; auditory startle response habituation and pre-pulse inhibition of startle on postnatal days 23/24 and 60/61; learning and memory on postnatal days 23/24 and 61/62), while a sixth pup was sacrificed on postnatal day 11 for examination of the brain. In all litters except two in the group receiving a dose of 3 mg/kg bw per day, a different pup was allocated to each behavioural test. Physical development of the pups was assessed by measurement of body weight. Sexual maturation of female pups was assessed by age at vaginal opening, and maturation of males was assessed by age at balano-preputial separation. Dams were sacrificed on postnatal day 21 and subjected to gross necropsy, abnormal tissues being retained for possible histopathological examination. Groups of 10 male and 10 female offspring were selected for detailed neuropathological examination and sacrificed on postnatal day 21 or postnatal day 65 ± 2 , respectively.

Treatment of dams with a dose of 0.1, 0.5 or 3 mg/kg bw per day had no adverse effect on clinical condition, survival, body-weight gain or food intake during gestation and lactation, gestation length, macroscopic necropsy findings or brain weights. There was no evidence for neurotoxicity in the dams, according to functional observational battery assessments. There was no effect of treatment on mean implantation rate, litter size or on mean pup weights on postnatal day 1. At 3 mg/kg bw per day, all offspring in six litters showed signs of poor general condition or retarded development during early lactation. Three affected litters at 3 mg/kg bw per day and one litter at 0.5 mg/kg bw per day were killed on postnatal days 2–4 for reasons of animal welfare. In addition to these deaths of entire litters during early lactation, there was an increase in pup mortality among litters that survived to weaning in the group receiving a dose of 3 mg/kg bw per day: The number of pups found dead or that were killed up to postnatal day 21 was 15, 11, 24 and 44 for the control group and the groups receiving the low, intermediate and highest dose, respectively. Background data on controls from five studies in which littering took place between October 2000 and September 2002 showed that up to one litter was found dead or killed for reasons of animal

welfare and a range of 10 to 33 pups were missing, found dead or killed among litters surviving to weaning at postnatal day 21. At 3 mg/kg bw per day, body-weight gains of male and female offspring during postnatal days 1–4 were about 30% lower than those of controls, but the differences did not attain statistical significance. Thereafter, weight gains were comparable or only marginally inferior to those of controls, such that overall gains during postnatal days 1–21 were about 10% lower than those of controls. Direct dosing of offspring during postnatal days 11–21 did not adversely affect body-weight gains and no clinical signs were observed that were considered to be related to treatment. Among offspring maintained until postnatal day 65 ± 2 , there was no effect of treatment on general clinical condition, survival, body-weight gains during postnatal days 21–63 or age at attainment of sexual maturation. Treatment-related differences in the functional performance of offspring were limited to the groups receiving a dose of 3 mg/kg bw per day, before weaning. On postnatal day 4, males and female offspring tended to be less active than the controls, as shown by lower values for maximum pivoting angle, maximum distance travelled and number of sections entered in the arena. Although these differences did not achieve statistical significance, the consistency between all three measures, and between males and females, did indicate an effect of treatment. Reduced arena activity was also observed in males and females on postnatal day 21. There was no evidence of any treatment-related effect in offspring performance during post-weaning observations and functional testing, including monitoring of learning and memory, auditory startle response and sexual maturation. There was no effect of treatment on findings made on macroscopic necropsy or on brain weights of selected offspring killed on postnatal days 11, 21 or 65 ± 2 , or on brain length and width on postnatal days 21 and 65 ± 2 . Also, there was no effect of treatment on histopathological findings or brain morphometry for selected offspring killed on postnatal days 21 or 65 ± 2 .

Treatment with dimethoate at a dose of up to 3 mg/kg bw per day, the highest dose tested, was not associated with any selective developmental neurotoxicity. The NOAEL for functional development of the nervous system and systemic toxicity in the offspring was 0.5 mg/kg bw per day, on the basis of developmental delay in some functional parameters and increased pup mortality at a dose of 3 mg/kg bw per day, after maternal treatment by oral gavage from day 6 of gestation until postnatal day 10, and direct treatment of the offspring on postnatal days 11–21 (Myers, 2001c).

2. Effects on cholinesterase activity in rats

2.1 Study of cholinesterase activity after single and repeated doses of dimethoate

The purpose of this study, which complied with UK GLP regulations 1999, European Commission Directive 1999/11/EC and OECD GLP principles 1997, was to assess the effect of single and/or repeated doses of dimethoate (purity, 99.1%) on plasma, erythrocyte and brain cholinesterase activities in pregnant female Sprague-Dawley CrI:CD(SD)IGS BR rats and their pre-term fetuses, in pre-weaning offspring and in young adult rats. Recovery of cholinesterase activity was also assessed in young adults 39 days after cessation of treatment with repeated doses.

In trial A, groups of 19 mated female rats (aged 10–11 weeks) received dimethoate at a dose of 0, 0.1, 0.5 or 3 mg/kg bw per day by gavage in water. Ten females per group were treated from day 6 of gestation until postnatal day 10, while the remaining nine females were treated on days 6–20 of gestation. For the females treated until day 20 of gestation, eight dams per group were killed 3 h after dosing on that day; data on litters were assessed and cholinesterase activity was determined for maternal and fetal plasma, erythrocytes and

brain. For the females that were allowed to litter and were treated until postnatal day 10, offspring in eight litters per group were treated on postnatal days 11–21 inclusive, in order to assess effects on survival, body-weight gain and cholinesterase activity. Selected offspring from these litters were killed on postnatal day 4, 21 or 60, and cholinesterase activity was determined for plasma, erythrocytes and brain. Time of parturition and duration of gestation were noted. Body weight was noted several days during gestation and after parturition, until postnatal day 21.

In trial B, a group of eight pregnant female rats was not treated throughout the study. Of the offspring of these females, one male and one female per litter were given dimethoate as a single dose of 0, 0.1, 0.5 or 3 mg/kgbw by gavage in water on postnatal day 11. Eight male and eight female offspring per group (one male and one female from each litter) were killed 2 h after dosing and cholinesterase activity was determined for plasma, erythrocytes and brain.

In trial C, groups of 16 male and 16 female naive adult rats (aged 7–8 weeks) received dimethoate at a dose of 0, 0.1, 0.5 or 3 mg/kgbw per day by gavage in water. Eight males and eight females per group were treated for one day and were killed 2 h after dosing, while the remaining eight males and eight females per group were treated for 11 consecutive days, and killed 2 h after the last dose. In each case, cholinesterase activity was determined for plasma, erythrocytes and brain.

Blood samples were collected from the retro-orbital sinus (for pups on postnatal day 21 and adults), umbilical cord (fetuses on day 20 of gestation) or by decapitation (pups on postnatal day 4 or 11). Blood samples were taken 3 h after dosing on day 20 of gestation, 4 h after dosing on postnatal day 4, and 2 h after dosing on postnatal days 11–21. Fetal blood samples from day 20 of gestation were pooled for each litter. Plasma samples and erythrocyte haemolysates were stored at -80°C until analysis. Brains were removed immediately after sacrifice, weighed and frozen in liquid nitrogen. Fetal brains from day 20 of gestation were pooled for each litter because the range-finding study showed no difference in cholinesterase activity between the sexes. Cholinesterase activity was determined according to a modified Ellman method (Environmental Protection Agency, 1999). Erythrocyte cholinesterase activity was measured using 6-6'-dithiodinicotinic acid, plasma and brain activity was measured using 5,5-dithio-bis(2-nitrobenzoic acid) as colour component.

On day 20 of gestation, the dams were sacrificed immediately after blood sampling, and brains were collected. The reproductive tract was examined for the following parameters: number of corpora lutea, number of implantation sites, number of resorption sites, and number and distribution of fetuses in each horn. Fetuses were weighed and sexed, and sacrificed on a cool plate at 0°C . The brains of the fetuses were then sampled. The dams were thoroughly examined macroscopically and specimens of any abnormal tissue were retained. All pups (except those culled on postnatal day 4) were examined macroscopically. In order to gain experience in a specific method of whole body perfusion fixation of tissue, and in precise sectioning of brains from young adult rats, five offspring were killed on postnatal day 61, perfused and the brain embedded, sectioned and subjected to examination by light microscopy.

There were no clinical signs that were considered to be related to treatment and no deaths at any dose. There was no adverse effect of treatment on weight gain of adult males

and females, weight change of dams during gestation and lactation or macroscopic findings at necropsy. Litter data on day 20 of gestation and body weight, weight gain and survival of offspring up to postnatal day 11 were unaffected by treatment. There was no effect of direct treatment on the growth or survival of the offspring.

In trial A, on day 20 of gestation, plasma and erythrocyte cholinesterase activity was statistically significantly inhibited at 3 mg/kg bw per day in dams (56% and 42% of control value) and in fetuses (57% and 69% of control value). Brain cholinesterase activity was statistically significantly inhibited in dams at 0.5 and 3 mg/kg bw per day (90% and 40% of control value) and in fetuses at 0.1, 0.5 and 3 mg/kg bw per day (88%, 90% and 67% of control value). In pups at postnatal day 4, plasma cholinesterase activity was statistically significantly inhibited at 0.5 and 3 mg/kg bw per day in females (92% and 90% of control value), while erythrocyte cholinesterase activity was statistically significantly inhibited at 3 mg/kg bw per day in males only (83% of control value). Brain cholinesterase activity was statistically significantly inhibited in males at 0.1, 0.5 and 3 mg/kg bw per day (90%, 92% and 87% of control value). In pups on postnatal day 21, plasma cholinesterase activity was statistically significantly inhibited at 3 mg/kg bw per day in both sexes (61% of control value in males; 62% in females), while erythrocyte cholinesterase activity was statistically significantly inhibited at 3 mg/kg bw per day in both sexes (41% of control value in males; 35% in females) and at 0.5 mg/kg bw per day in females (77% of control value). Brain cholinesterase activity was statistically significantly inhibited in males at 0.1, 0.5 and 3 mg/kg bw per day (96%, 87% and 55% of control value) and in females at 0.5 and 3 mg/kg bw per day (88% and 58% of control value). Offspring killed on postnatal day 60 (i.e. 39 days after the end of repeated dosing) showed complete recovery of cholinesterase activity; the statistically significant inhibition of brain cholinesterase activity in females at 0.5 and 3 mg/kg bw per day (96% and 96% of control value) was considered to be not biologically significant.

In trial B, in offspring treated once on postnatal day 11, plasma and brain cholinesterase activity was statistically significantly inhibited at 3 mg/kg bw per day in males (81% and 83% of control value). In females, inhibition of plasma, erythrocyte and brain cholinesterase activities was seen at the same dose (82%, 74% and 82% of control value), differences attaining statistical significance for brain cholinesterase activity only. The statistically significant inhibition of brain cholinesterase activity in males at 0.5 mg/kg bw per day (95% of control value) was considered to be not biologically significant.

In trial C, treatment of naive adult animals with a single dose of 3 mg/kg bw per day was associated with inhibition of plasma, erythrocyte and brain cholinesterase activity in males (81%, 83% and 88% of controls) and in females (87%, 73% and 86% of controls); differences attained statistical significance for all parameters in males and for erythrocyte and brain cholinesterase activity in females. The statistically significant inhibition of brain cholinesterase activity in males at 0.5 mg/kg bw per day (96% of control value) was considered to be not biologically significant. Treatment of naive adults with 11 consecutive doses of dimethoate at 3 mg/kg bw per day was associated with inhibition of plasma, erythrocyte and brain cholinesterase activity in males (63%, 42% and 53% of controls) and in females (79%, 37% and 42% of controls); differences attained statistical significance for all parameters, except plasma cholinesterase activity in females. At 0.5 mg/kg bw per day, there was a slight, statistically non-significant inhibition of plasma and erythrocyte cholinesterase activity in males (88% and 83% of controls), while brain

cholinesterase activity was statistically significantly inhibited in males and females (90% and 87% of controls).

The NOAEL for dimethoate given as a single dose was 0.5 mg/kg bw per day on the basis of a statistically significant inhibition of brain cholinesterase activity in pre-weaning rats (17–18%) and in young adults (12–14%) and a significant inhibition of erythrocyte cholinesterase activity in pre-weaning females (26%) and in young adult females (27%) at 3 mg/kg bw per day.

The NOAEL for dimethoate given as repeated doses was 0.1 mg/kg bw per day on the basis of a slight, statistically significant inhibition (10–13%) of brain cholinesterase activity in pregnant rats, pups on postnatal day 21 and young adult rats, and a statistically significant inhibition (23%) of erythrocyte cholinesterase activity in female pups on postnatal day 21 at ≥ 0.5 mg/kg bw per day. The slight, statistically significant depression (10–12%) of brain cholinesterase activity at 0.1 and 0.5 mg/kg bw per day in fetuses and in male pups aged 4 days was considered to be of doubtful biological significance, since there was no dose–response relationship (Myers, 2001a).

2.2 Dose-finding study preliminary to study of developmental neurotoxicity

Groups of 15 pregnant female Crl:CD BR rats (aged 10–11 weeks) were given dimethoate (purity, 99.1%) at a dose of 0, 0.2, 3 or 6 mg/kg bw per day by gavage in water. This study did not comply with GLP as it was not audited by quality assurance (QA). Ten females per group were treated from day 6 of gestation until postnatal day 10 and the remaining five females per group were treated on days 6–20 of gestation. The females treated until day 20 of gestation were killed 3 h after dosing on that day; data on litters were assessed and cholinesterase activity was determined in maternal and fetal plasma, erythrocytes and brain. For the females that were allowed to litter and were treated until postnatal day 10, two male and two female offspring per litter were treated on postnatal days 11–21, in order to assess effects on survival, weight gain and cholinesterase activity in plasma, erythrocytes and brain. The treated offspring were killed 2 h after dosing on postnatal day 21 and cholinesterase activity was determined. The remaining offspring in each litter were not treated and acted as within-litter “controls” for comparison of growth and survival among siblings. Dams and untreated offspring were killed on or shortly after postnatal day 21. In order to gain experience in gravity perfusion and histological sectioning of brains from pups on postnatal day 21 to allow examination of specific regions of the brain, four untreated pups were killed on postnatal day 21, perfused and the brain embedded, sectioned and subjected to examination by light microscopy. For cholinesterase measurements, blood samples were taken from the retro-orbital sinus (adults and pups) and umbilical cord (fetuses) 2–3 h after dosing. The fetal blood samples from day 20 of gestation were pooled for each litter. The resulting plasma samples and erythrocyte haemolysates were stored at -80°C until analysis. The brains were removed immediately after sacrifice, weighed and frozen in liquid nitrogen and stored until analysis. The fetal brains from day 20 of gestation were pooled for males and females fetuses for each litter. Cholinesterase activity was determined according to a modification of the Ellman method (Environmental Protection Agency, 1996).

In the dams, no deaths occurred throughout the study, and no differences in clinical signs were detected between groups. Body weight was affected at a dose of 6 mg/kg bw per day and 3 mg/kg bw per day. A statistically significantly reduced maternal body-weight gain

was observed on days 10–20 of gestation at 6 mg/kg bw per day and at 3 mg/kg bw per day. Lower (but not statistically significantly different from control) mean body weight that persisted until postnatal day 17 was noted at both doses. Food consumption was not affected by treatment. No treatment-related findings were noted at necropsy on day 20 of gestation or postnatal day 21. In the dams killed on day 20 of gestation, cholinesterase activity was inhibited at 3 and at 6 mg/kg bw per day in plasma (75% and 43% of control value), in erythrocytes (22% and 15% of control value) and in brain (25% and 12% of control value).

Litter parameters on day 20 of gestation and the number of implantation sites were unaffected by the treatment. In the fetuses, plasma cholinesterase activity was inhibited at 0.2, 3 and 6 mg/kg bw per day in males (80%, 25% and 21% of control value) and females (88%, 34% and 27% of control value). Erythrocyte cholinesterase activity was inhibited in males at 3 and 6 mg/kg bw per day (30% and 13% of control value) and in females at 0.2, 3 and 6 mg/kg bw per day (70%, 18% and 4% of control value). Brain cholinesterase activity was inhibited at 3 and 6 mg/kg bw per day in males (78% and 65% of control value) and females (76% and 58% of control value). Total litter size on postnatal day 1 was not affected by treatment. However, increased postnatal mortality was observed at 6 mg/kg bw per day. Two normal-sized litters died on postnatal day 2 and postnatal day 5, while another dam lost 4/14 offspring on postnatal day 4. This resulted in reductions in live litter size and offspring viability index. Group mean body weight and body-weight gain for the offspring was reduced during postnatal days 1–11 at 6 mg/kg bw per day. Direct dosing of offspring on postnatal days 11–21 did not affect offspring survival, body weight or body-weight gain at any dose. On postnatal day 21, cholinesterase activity was inhibited at 3 and at 6 mg/kg bw per day in plasma (61% and 40% of control value in males, 60% and 40% of control value in females, respectively), in erythrocytes (40% and 30% of control value in males, 35% and 20% of control value in females, respectively) and in brain (55% and 45% of control value in males, 58% and 34% of control value in females, respectively).

The NOAEL was 0.2 mg/kg bw per day for dams and their offspring under the conditions of this study, on the basis of reduced body-weight gain in dams and inhibition of erythrocyte and brain cholinesterase activity in dams, fetuses and pups at 3 mg/kg bw per day. The slightly decreased erythrocyte cholinesterase activity in female fetuses at 0.2 mg/kg bw per day was considered to be not biologically significant, since there were no effects on brain cholinesterase activity nor any effects in males (Myers, 2001b).

2.3 Study of developmental neurotoxicity

Groups of 24 pregnant female Crl:CD BR rats (aged 10–11 weeks) were given dimethoate (purity, 99.1%) by oral gavage in water at a dose of 0, 0.1, 0.5 or 3 mg/kg bw per day from day of gestation until postnatal day 10; offspring received the same doses by oral gavage on postnatal days 11–21. This study complied with UK GLP regulations 1999, European Commission Directive 1999/11/EC, OECD GLP principles 1997, and EPA guideline OPPTS 870.6300. Clinical observation was performed on all dams at least twice daily throughout the study. Body weight and food consumption were recorded several times throughout the study; parturition and duration of gestation were noted. Ten dams per group were subjected to a detailed clinical examination and open arena observations on days 12 and 18 of gestation, and on postnatal days 4 and 10. On postnatal day 4, litters were culled to eight pups each (four males and four females when possible). Five pups from each litter

were allocated to undergo further functional investigations (motor activity on postnatal days 13, 17, 22 and 59; auditory startle response habituation and pre-pulse inhibition of startle on postnatal days 23/24 and 60/61; learning and memory on postnatal days 23/24 and 61/62), while a sixth pup was sacrificed on postnatal day 11 for examination of the brain. In all litters except two in the group receiving a dose of 3 mg/kg bw per day, a different pup was allocated to each behavioural test. Physical development of the pups was assessed by measurement of body weight. Sexual maturation of female pups was assessed by age at vaginal opening, and maturation of males was assessed by age at balano-preputial separation. Dams were sacrificed on postnatal day 21 and subjected to gross necropsy, abnormal tissues being retained for possible histopathological examination. Groups of 10 male and 10 female offspring were selected for detailed neuropathological examination and sacrificed on postnatal day 21 or postnatal day 65 ± 2 , respectively.

Treatment of dams with a dose of 0.1, 0.5 or 3 mg/kg bw per day had no adverse effect on clinical condition, survival, body-weight gain or food intake during gestation and lactation, gestation length, macroscopic necropsy findings or brain weights. There was no evidence for neurotoxicity in the dams, according to functional observational battery assessments. There was no effect of treatment on mean implantation rate, litter size or on mean pup weights on postnatal day 1. At 3 mg/kg bw per day, all offspring in six litters showed signs of poor general condition or retarded development during early lactation. Three affected litters at 3 mg/kg bw per day and one litter at 0.5 mg/kg bw per day were killed on postnatal days 2–4 for reasons of animal welfare. In addition to these deaths of litters during early lactation, there was an increase in pup mortality among litters that survived to weaning in the group receiving a dose of 3 mg/kg bw per day: The number of pups found dead or that were killed up to postnatal day 21 was 15, 11, 24 and 44 for the control group and the groups receiving the low, intermediate and highest dose, respectively. Background data on controls from five studies in which littering took place between October 2000 and September 2002 showed that up to one litter was found dead or killed for reasons of animal welfare and a range of 10 to 33 pups were missing, found dead or killed among litters surviving to weaning at postnatal day 21. At 3 mg/kg bw per day, body-weight gains of male and female offspring during postnatal days 1–4 were about 30% lower than those of controls, but the differences did not attain statistical significance. Thereafter, weight gains were comparable or only marginally inferior to those of controls, such that overall gains during postnatal days 1–21 were about 10% lower than those of controls. Direct dosing of offspring during postnatal days 11–21 did not adversely affect body-weight gains and no clinical signs were observed that were considered to be related to treatment. Among offspring maintained until postnatal day 65 ± 2 , there was no effect of treatment on general clinical condition, survival, body-weight gains during postnatal days 21–63 or age at attainment of sexual maturation. Treatment-related differences in the functional performance of offspring were limited to the groups receiving a dose of 3 mg/kg bw per day, before weaning. On postnatal day 4, males and female offspring tended to be less active than the controls, as shown by lower values for maximum pivoting angle, maximum distance travelled and number of sections entered in the arena. Although these differences did not achieve statistical significance, the consistency between all three measures, and between males and females, did indicate an effect of treatment. Reduced arena activity was also observed in males and females on postnatal day 21. There was no evidence of any treatment-related effect in offspring performance during post-weaning observations and functional testing, including monitoring of learning and memory, auditory startle response and sexual maturation. There was no effect of treatment on findings made on macroscopic necropsy or on brain weights of selected offspring killed on postnatal days 11, 21 or 65 ± 2 , or on brain

length and width on postnatal days 21 and 65 ± 2 . Also, there was no effect of treatment on histopathological findings or brain morphometry for selected offspring killed on postnatal days 21 or 65 ± 2 .

Treatment with dimethoate at a dose of up to 3 mg/kg bw per day, the highest dose tested, was not associated with any selective developmental neurotoxicity. The NOAEL for functional development of the nervous system and systemic toxicity in the offspring was 0.5 mg/kg bw per day, on the basis of developmental delay in some functional parameters and increased pup mortality at a dose of 3 mg/kg bw per day, after maternal treatment by oral gavage from day 6 of gestation until postnatal day 10, and direct treatment of the offspring on postnatal days 11–21 (Myers, 2001c).

3. Studies on metabolites

3.1 *Effects of dimethoate, omethoate and four metabolites on erythrocyte cholinesterase activity*

In a study that was performed according to UK GLP regulations for 1999, European Commission Directive 1999/11/EC and OECD GLP principles for 1997, groups of five male Crl:CD(SD)IGS BR rats (aged 8 weeks) received one of the following treatments: (i) dimethoate (purity, 99.5%) in a single dose of 30 mg/kg bw; or (ii) four metabolites (metabolite I: *O*-desmethyl omethoate potassium salt (purity, 98.6%); metabolite II: *O*-desmethyl omethoate carboxylic acid potassium salt (purity, 89.5%); metabolite III: *O*-desmethyl-*N*-desmethyl omethoate potassium salt (purity, 96.4%); metabolite IV: *O*-desmethyl-isodimethoate dicyclohexylammonium salt (purity, 98.2%) as a single dose of 30 mg/kg bw; or (iii) omethoate (purity, 96.3%) as a single dose of 5 mg/kg bw, given by oral gavage in water.

Detailed clinical observations were made before dosing, at return to home cage, after dosing of the group and every 2 h for the first 24 h after dosing of the group. Body-weight was recorded before dosing, on the day of treatment and twice weekly thereafter. Blood samples for determination of erythrocyte cholinesterase activity were taken before dosing (after a fast of 8 h), at about 2.5 h after dosing and 24 h after dosing (again after 8 h fasting). All animals were killed at termination and examined macroscopically.

No mortalities, no clinical signs of toxicity and no effects on body-weight gain were observed in any group during 14 days. No treatment-related findings were noted at necropsy. Markedly and statistically significantly lower erythrocyte cholinesterase activity was seen for animals receiving dimethoate at a dose of 30 mg/kg bw (47% and 60% of the pre-dosing value at 2.5 h and 24 h after treatment, respectively) or omethoate at a dose of 5 mg/kg bw (26% and 66% of the pre-dosing value at 2.5 h and 24 h after treatment, respectively). For animals receiving metabolites I, II or IV, erythrocyte cholinesterase activity decreased slightly at 2.5 h after dosing (81%, 75% or 72% of pre-dosing value) and at 24 h after dosing (84%, 79% or 80% of the pre-dosing value). No effect on erythrocyte cholinesterase activity was seen in animals receiving metabolite III (Brennan, 2001).

3.2 *Peak effect of omethoate on clinical signs*

Groups of five male and five female Wistar Crl Glx Brl Han:WI rats (aged about 7 weeks) received omethoate (purity, 96.5%) by oral gavage in water as a single dose of 0, 5, 10 or 15 mg/kg bw, in a study that did not comply with GLP as it was not audited by QA.

Detailed clinical observations (including abbreviated FOB) were made before treatment, immediately after dosing and at 1, 2, 4, 7 and 24 h thereafter. Body-weight was determined once before dosing. Blood samples for the determination of plasma and erythrocyte cholinesterase activity were taken from the retro-orbital sinus 8 h after dosing and before necropsy (24 h after dosing). Brains were removed immediately after sacrifice for determination of cholinesterase activity; no further pathology examinations were carried out. Cholinesterase activity was determined according to a modified Ellman method (Environmental Protection Agency, 1996). Erythrocyte cholinesterase activity was measured using the colour component DNTA, while plasma and brain cholinesterase activity was measured using DNTB.

No animals died during the study. Major clinical signs of cholinergic stimulation that occurred in a dose-dependent and time-dependent manner in the treated groups were tremors, impairment of gait, loss of pupillary reflex, irregular respiration, and frequent chewing. The time of peak effect was 2–4 h at the lowest dose and 1–4 h at the intermediate and highest dose. Plasma cholinesterase activity was statistically significantly inhibited in males at 5, 10 and 15 mg/kg bw (48%, 41% and 36% of control value after 8 h; 68%, 52% and 47% of control value after 24 h) and in females at 10 and 15 mg/kg bw (48% and 53% of the control value after 8 h; 52% and 45% of the control value after 24 h). Erythrocyte cholinesterase activity was statistically significantly inhibited in both sexes at 5, 10 and 15 mg/kg bw (males: 44%, 35% and 26% of the control value after 8 h, and 55%, 37% and 28% of the control value after 24 h; females: 41%, 32% and 31% of the control value after 8 h, and 51%, 36% and 30% of the control value after 24 h). Brain cholinesterase activity was inhibited in both sexes at 5, 10 and 15 mg/kg bw (males: 59%, 44% and 41% of the control value; females: 60%, 56% and 44% of the control value), the differences attained statistical significance for males at the highest dose and for females at the intermediate and highest doses (Mellert et al., 2002a).

3.3 *Inhibition of cholinesterase activity by omethoate*

Groups of 10 male Wistar Crl Glx Brl Han:WI rats (aged about 7 weeks) received omethoate (purity, 96.5%) as a single dose of 0, 0.25, 0.5, 0.75 or 1.5 mg/kg bw by oral gavage in water, in a study that did not comply with GLP as it was not audited by QA. A check for moribund or dead animals was made twice daily, and body weight was determined once before dosing. Blood samples for determination of plasma and erythrocyte cholinesterase activities were taken from the retro-orbital sinus 2.5 h after dosing and before necropsy (24 h after dosing). Brains were removed immediately after sacrifice for the determination of cholinesterase activity; no further pathology examinations were carried out. Cholinesterase activity was determined according to a modified Ellman method (Environmental Protection Agency, 1996). Erythrocyte cholinesterase activity was measured using the colour component DNTA, while plasma and brain cholinesterase activity was measured using DNTB.

No animals died and no abnormal clinical signs were detected during the study. Plasma cholinesterase activity was statistically significantly inhibited at 0.75 and 1.5 mg/kg bw at 2.5 h after dosing (81% and 65% of control value). Erythrocyte cholinesterase activity was statistically significantly inhibited at 0.25, 0.5, 0.75 and 1.5 mg/kg bw at 2.5 h after dosing (87%, 73%, 62% and 44% of the control value) and at 0.75 and 1.5 mg/kg bw at 24 h after dosing (86% and 75% of the control value). Brain cholinesterase activity was marginally inhibited at 0.5 and 0.75 mg/kg bw and moderately inhibited at 1.5 mg/kg bw

(87%, 89% and 69% of control value); the differences did not attain statistical significance (Mellert et al., 2002b).

Comments

The LD₅₀ of dimethoate administered orally was about 314–600 mg/kg bw in rats and 150 mg/kg bw in mice.

Acute neurotoxicity was studied in rats given dimethoate at a single dose of 0, 2, 20 or 200 mg/kg bw by gavage, after preliminary studies had shown that peak effects for clinical signs occur about 2 h after dosing. Abnormal clinical signs and effects on FOB parameters were seen at the highest dose, mainly during the first 2 days after treatment and disappeared by day 7. The NOAEL was 2 mg/kg bw on the basis of the absence of pupil response at ≥20 mg/kg bw. Cholinesterase activity was not analysed in this study.

In a study of acute neurotoxicity in rats given dimethoate in the diet as a single doses of 0, 1, 2, 3 or 15 mg/kg bw, no clinical signs and no effects on FOB parameters were observed. A statistically significant inhibition of cholinesterase activity in erythrocytes of males (29%) and in the brain cortex of females (11%) was observed at ≥3 mg/kg bw.

In a special study designed to assess effects on cholinesterase activity, pre-weaning rats (aged 11 days) and young adult rats (aged 7–8 weeks) received dimethoate as a single dose of 0, 0.1, 0.5 or 3 mg/kg bw by gavage. There was no difference in susceptibility between pre-weaning and young adult rats. A statistically significant inhibition of brain cholinesterase activity in pre-weaning rats (17–18%) and in young adult rats (12–14%) and of erythrocyte cholinesterase activity in pre-weaning and young adult female rats (26–27%) was observed at 3 mg/kg bw.

The Meeting concluded that the overall NOAEL for acute effects on cholinesterase activity was 2 mg/kg bw.

The Meeting also considered a number of studies in human volunteers, which indicated that single or repeated oral doses of dimethoate of up to 0.2 mg/kg bw did not induce clinical effects or inhibit cholinesterase activity in the blood. It was concluded that these studies were not conducted according to current standards (no details on study design, e.g. age and sex of individual volunteers, were given and no raw data were provided). Therefore, the Meeting considered that the studies in humans were only supportive for setting the acute RfD.

The Meeting also reviewed new studies that were not relevant to the establishment of an acute RfD. In a study of neurotoxicity, rats received dimethoate in the diet at concentrations of 0, 1, 50 or 125 mg/kg. The NOAEL for systemic toxicity and neurotoxicity was 1 mg/kg (equal to 0.06 mg/kg bw per day) on the basis of inhibition of erythrocyte cholinesterase activity (34–49%) and small faeces at ≥50 mg/kg (equal to ≥3.22 mg/kg bw per day).

In a special study designed to assess effects on cholinesterase activity, pregnant rats, pre-weaning rats and young adult rats received dimethoate by gavage as repeated doses at 0, 0.1, 0.5 or 3 mg/kg bw per day. The NOAEL was 0.1 mg/kg bw per day on the basis of a

consistent, statistically significant inhibition of brain cholinesterase activity (10–13%) in pregnant, pre-weaning and young adult rats and of erythrocyte cholinesterase activity (23%) in pre-weaning female pups at ≥ 0.5 mg/kg bw per day.

In a study of developmental neurotoxicity, pregnant rats received dimethoate by gavage at a dose of 0, 0.1, 0.5 or 3 mg/kg bw per day from day 6 of gestation to postnatal day 10, and their offspring received the same doses by gavage on postnatal days 11–21. The NOAEL for functional development of the nervous system and systemic toxicity in the offspring was 0.5 mg/kg bw per day on the basis of developmental delay in some functional parameters and increased pup mortality at a dose of 3 mg/kg bw per day. The Meeting considered these effects to be of no relevance for setting the acute RfD, since they would not be expected to occur after a single exposure, and concluded that the new studies supported the current ADI of 0–0.002 mg/kg bw.

Toxicological evaluation

After considering the previous evaluations of dimethoate and the new data submitted, the Meeting established an acute RfD of 0.02 mg/kg bw on the basis of the overall NOAEL of 2 mg/kg bw for cholinesterase inhibition in studies in rats, and a safety factor of 100. This acute RfD was supported by the NOAEL of about 0.2 mg/kg bw per day in studies in volunteers receiving single or repeated doses, which were evaluated by the 1996 JMPR.

The Meeting recognized that it might be possible to refine this acute RfD on the basis of further characterization of the effects caused by dimethoate.

Estimate of acute reference dose

0.02 mg/kg bw

Studies that would provide information useful for continued evaluation of the compound

- Further observations in humans
- The two-generation study of reproductive toxicity (available in abbreviated form at the 2003 Meeting)

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