PHOSMET (addendum)

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Explanation

Phosmet (*O,O*-dimethyl *S*-phthalimidomethyl phosphorodithioate) is an insecticide and acaricide that acts by inhibiting acetylcholinesterase activity. Phosmet was evaluated previously by the JMPR in 1978, 1979, 1994 and 1998 (Annex 1, references 30, 32, 71, 83). An ADI of 0–0.01 mg/kg bw was set in 1994. In 1998, an acute reference dose (RfD) of 0.02 mg/kg bw was set, based on a NOAEL of 2 mg/kg bw per day in a study of developmental toxicity in rabbits. By request of a WHO Member State and the European Union, the acute RfD for phosmet was reviewed by the present Meeting in 2003, instead of in 2004 as originally scheduled. A recent study in human volunteers and additional information on the study of developmental toxicity in rabbits were reviewed by the current Meeting.

Evaluation for acute reference dose

The Meeting considered the new data and reviewed previously submitted information of relevance to the establishment of an acute RfD.

1. Developmental toxicity

Rats

In reviewing a study of developmental toxicity in rats (Hodge, 1991), the 1994 Joint Meeting (Annex 1, reference 73) concluded that: ". . . 5 mg/kg bw per day was the NOAEL for maternal toxicity. As no teratogenic or fetotoxic effects were seen, the NOAEL for developmental toxicity was 15 mg/kg bw per day." In this study, groups of 24 mated female Wistar rats received phosmet (purity, 96.4%) at a dose of 0, 5, 10 or 15 mg/kg bw per day by gavage. Signs typical of cholinergic toxicity were seen in dams at 15 mg/kg bw per day, with reduced maternal body-weight gain evident at 10 and 15 mg/kg bw per day.

In a published paper (Staples, 1976), it was reported that the administration of phosmet at a dose of up to 30 mg/kg bw per day to groups of CD rats did not cause any

fetotoxicity. The NOAEL for maternal toxicity was reported to be 10 mg/kg bw per day. The 1994 JMPR was unable to set a NOAEL, as no concurrent control group was used

Rabbits

In a range-finding study of developmental toxicity, groups of 10 New Zealand white rabbits were given phosmet (purity, 96.4%) at a dose of 0, 5, 10 or 15 mg/kg bw per day by gavage. The fetuses were examined only for external malformations and cleft palate. Maternal toxicity was seen at 15 mg/kg bw per day, but no malformations were reported (Pinto, 1991).

Groups of 20 inseminated female New Zealand white rabbits were given phosmet (purity, 96.4%) at a dose of 0, 2, 5 and 15 mg/kg bw per day in corn oil by gavage on days 7–19 of gestation (insemination was performed on day 1). The animals were observed for clinical signs, and body weight and food consumption were recorded at regular intervals. On day 30 of gestation, all surviving animals were sacrificed and uteri were examined for live fetuses and intra-uterine deaths. All fetuses were weighed, sacrificed, and examined for external and visceral abnormalities, sexed, eviscerated and stained for skeletal examination. Assays for cholinesterase activity were not performed in this study. The study complied with good laboratory practice (GLP) and OECD test guideline 414 (1981).

A total of six rabbits died or were sacrificed: two at 15 mg/kg bw per day; three at 5 mg/kg bw per day and one at 2 mg/kg bw per day. One of the animals receiving the highest dose was sacrificed after showing signs of cholinergic toxicity. Maternal toxicity was evident at 15 mg/kg bw per day (Table 1), with reduced body-weight gain during the period of treatment (13.5 g versus 51.8 g in controls) and occasional clinical signs typical of acetylcholinesterase inhibition (e.g. salivation). Food consumption was reduced by about 10% at 15 g/kg bw per day at the start of treatment. There was no evidence of maternal toxicity at 5 mg/kg bw per day. Complete resorption of a litter and a reduction in litter size at 2 mg/kg bw per day were not dose-related and were considered to be chance findings. Increases in total external and visceral defects, delayed ossification and minor skeletal defects were evident at 5 and 15 mg/kg bw per day (Table 1), but overall rates were reported to be within the ranges for contemporary historical controls. There was no clear evidence for a treatment-related increase in any individual variation or malformation. The only statistically significant increase in an individual variation was in extreme flexion of the forepaw at 5 mg/kg bw per day, but this was not reproduced at 15 mg/kg bw. A similar absence of dose–response relationship was seen in the sites of altered ossification at 5 mg/kg bw per day (Table 1); a dose–response relationship would be expected because approximately 90% of the administered dose of phosmet was absorbed at 25 mg/kg bw per day. There is no consensus on whether alterations in ossification of fetal bones are a specific effect that can be produced by a single dose, or whether they are secondary to maternal toxicity, or both of these. It was considered that the fetal effects seen at 5 mg/kg bw per day were minimal and not clearly related to a single dose of phosmet. The parental and fetal toxicity seen at 15 mg/kg bw per day were considered to be related to inhibition of cholinesterase activity (Moxon, 1991).

2. Observations in humans

Groups of human volunteers were given phosmet (purity, 96%) or placebo (lactose BP) in a study that was approved by an ethics committee, used prior informed consent, and complied with the declaration of Helsinki. The study was a double-blind, randomized,

Table 1. Findings in rabbits given phosmet by gavage during days 7–19 of gestation

Parameter	Dose (mg/kg bw)				Historic controls[#] range (mean ± SD) as %
	0	2	5	15	
Maternal body-weight gain (g) on days 7–19	51.8	69.9	57.4	13.5	NR
Maternal body-weight gain (g) on days 7–10	–42.4	–51.3	–53.8	–72.9	NR
No. of pregnant dams	18	16	20	16	NR
No. of dams with live fetuses on day 30	18	14	17	15	NR
Maternal body-weight gain (g) on days 10–13	54.4	39.6	25.6	–2*	NR
Viable fetuses (total/mean per dam)	141/7.8	92/6.6	132/7.8	118/7.9	NR
Mean fetal weight (g)	42.8	46.6	42.2	41.6	NR
Fetuses with major defects (some have both skeletal and visceral) (%)	3 (2.1)	3 (3.3)	6 (4.5)	5 (4.2)	NR
No. of fetuses with major external or visceral defects (%)	3 (2.1)	2 (2.2)	64.5)	5 (4.2)	NR
No. of fetuses with minor external or visceral defects (%)	6 (4.3)	6 (6.5)	13 (9.8)	12 (10.2)	NR
No. of fetuses with major skeletal defects	2 (1.4)	1 (1.1)	1 (0.7)	1 (0.8)	NR
No. of fetuses with minor skeletal defects (mean %)	50 (35.4)	42 (45.6)	62* (46.9)	56* (47.4)	35.2–55.8 (42 ± 7.6)
No. of fetuses with skeletal variations (mean %)	136 (96.4)	91 (98.9)	121 (91.6)	111 (94.1)	NR
Partially ossified odontoid					
Fetus (%)	55 (39)	39 (42)	49 (37)	68** (57)	24–58 (39 ± 13)
Litter (%)	16 (88)	10 (63)	14 (70)	14 (87)	46–100 (80 ± 16)
Unossified fifth sternebra					
Fetus (%)	4 (3)	5 (5)	7 (5)	15** (13)	3–20 (9 ± 5)
Litter (%)	2 (10)	5 (31)	5 (25)	6 (37)	7–61 (28 ± 16)
Unossified sixth sternebrae					
Fetus (%)	8 (6)	5 (5)	8 (6)	11 (9)	0–8 (6 ± 2)[##]
Litter (%)	4 (22)	5 (31)	6 (30)	9* (56)	0–36 (29 ± 10)
Seventh transverse process partially ossified					
Fetus (%)	1	1	5 (4)	0	0–2.2 (0.4 ± 0.7)
Litter (%)			3 (15)		0–16.7 (3 ± 6)
Fully ossified second lumbar vertebrae					
Fetus (%)	0	0	6 (5)	0	0–5 (1.7 ± 1.9)
Litter (%)			3 (16)		0–21 (8 ± 7)
Bipartite sixth sternebrae					
Fetus (%)	1	2/2	5 (4)	1	0–5 (1.3 ± 1.4)
Litter (%)			3 (15)		0–31 (10 ± 9)
Extreme flexion of forepaw—fetus (%)	0	0	4 (4)	1	0
(fetal/litter incidence)—litter (%)			4* (15)		
Pes score (measure of foot ossification)	1.09	1.03	1.06	1.19*	1.03–2.0 (1.5 ± 0.5)

From Moxon (1991)
NR, not relevant
Nine studies conducted during 1990–1993, except ##, six studies conducted during 1990–1991
*$p < 0.05$; **$p < 0.01$

placebo-controlled protocol and subjects were selected, healthy men and women aged 18–50 years. Six men per group received a single oral dose of phosmet of 1, 2, or 4 mg/kg bw, and three men per group received placebo. Six women received a single oral dose of phosmet of 2 mg/kg bw, and three women received placebo. The test substances were administered orally in capsules with 150 ml of water, approximately 5 min after breakfast. The paired groups receiving phosmet or placebo were dosed sequentially: 1 mg/kg bw in men, 2 mg/kg bw in men, 4 mg/kg bw in men and then 2 mg/kg bw in women, groups receiving placebo being treated concurrently with groups receiving phosmet. Subjects were dosed in the sitting position and remained sitting until 4 h after dosing.

Subjects remained in the clinic for 48 h after dosing and returned for follow-up visits at 96 h and 168 h after dosing. Investigations included vital signs, 12-lead electrocardiogram (ECG), continuous single channel ECG (30 min before dosing until 4 h after dosing), urine analysis, haematology, clinical chemistry, oral temperature, adverse events and plasma and

erythrocyte cholinesterase activities. Samples for cholinesterase determination (three replicates per sample time) were taken on days –10, –8, –4, –2, –1 and –30 min (before dosing), and at 1, 2, 4, 8, 12, 24, 48, 96 and 168 h after dosing. All samples taken before dosing and those taken at 24, 48 and 96 h after dosing were done so in the morning, at the same time, if possible. One subject was removed from the study after breaking the study conditions, and was replaced.

The overall pattern of findings was similar in groups receiving placebo and in the groups receiving phosmet. Notable changes in values for an individual subject could usually be linked to results obtained before dosing. Reports of adverse events typical of cholinesterase inhibition were similar in groups receiving placebo and in groups receiving phosmet (Table 2). It should be noted that as part of the informed consent, subjects were told what potential side-effects might be expected. Two findings that were possibly related to treatment with phosmet were a dose-related decrease in mean serum glucose concentration and an inhibition of erythrocyte cholinesterase activity after 1 h and 4 h in males at 4 mg/kg bw (Table 3). There was no evidence for cholinesterase inhibition caused by treat-

Table 2. Summary of adverse events in volunteers given phosmet

	Dose (mg/kg bw)							
	0 (Placebo)		1		2		4	
	Males	Females	Males	Females	Males	Females	Males	Females
No. of subjects	10*	3	6	—	6	6	6	—
No. of subjects with adverse events (%)	2 (20%)	2 (67%)	0 (0%)	—	1 (17%)	3 (50%)	1 (17%)	—
No. of compound-related adverse events** (%)	1 (10%)	1 (33%)	0	—	1 (17%)	0	1 (17%)	—

From Cameron (1999)

* Includes subject who was removed, and the replacement

** Test compound-related adverse events are defined as those assessed by the investigator (before the study blind was broken) as having a potential relationship to administration of the test compound of "possibly related", "probably related" or "definitely related"

Table 3. Changes in erythrocyte cholinesterase activity (% relative to individual baseline value before dosing) in volunteers receiving phosmet

Time point (h after dosing)	Value given	Dose (mg/kg bw)				Linear trend
		0 (Placebo)	1	2	4	
Males						
1	Adjusted means*	4.39	0.37	3.04	–4.44	0.068
	(range)	(–8.7; +13.2)	(12.2; +10.4)	(–2.8; +8.4)	(–19.3; +21.1)	
2	Adjusted means*	2.51	3.25	–2.47	4.52	0.77
4	Adjusted means*	–0.65	1.53	–1.33	–6.87	0.093
	(range)	(–13.3; +13.2)	(–5.6; +7.2)	(–9.4; +4.7)	(–14.0; +1.1)	
8	Adjusted means*	10.51	2.33	–0.67	12.70	0.38
12	Adjusted means*	7.57	–4.57	2.24	9.10	0.23
24	Adjusted means*	7.50	–4.51	1.14	0.24	0.34
48	Adjusted means*	6.47	0.29	1.40	2.69	0.59
96	Adjusted means*	–2.93	–6.98	–4.41	–5.50	0.74
168	Adjusted means*	1.97	–5.11	5.00	0.44	0.78
1–168	Range	–17.3; +35.5	–17.2; +10.4	–14.9; +13.7	–19.4; +27.4	
Females						
1–168	Range	–12.4; +17.6	—	–16.2; +26.8	—	—

From Cameron (1999)

* Represented adjusted means from repeated measures (analysis of variance, ANOVA)

ment with phosmet in females. The changes in glucose concentrations in men treated with phosmet (relative to values before dosing) were within the range of values for men receiving placebo, and all values for men and women (4.3–6.0 mmol/l) were within the normal range. The maximum inhibition of erythrocyte cholinesterase activity in any individual, relative to their baseline value before dosing, was <20% (Table 3) and the overall patterns were similar in groups receiving placebo or phosmet. The Meeting concluded that there were no consistent, biologically significant effects of treatment with phosmet at doses of 4 mg/kg bw in men and 2 mg/kg bw in women. The NOAEL was 2 mg/kg bw, the highest dose tested in both sexes (Cameron, 1999).

3. Other issues

There are a number of other issues that might impact upon the use of the data from studies in human volunteers for setting an acute reference dose for phosmet.

The study in human volunteers (Cameron, 1999) only measured parameters that could be evaluated by non-invasive techniques, plus blood sampling for haematology, clinical chemistry and cholinesterase activities. It is necessary to confirm that there are no other effects that are critical to the evaluation of phosmet. In two studies there was an indication that brain acetylcholinesterase activity was inhibited to a greater extent than erythrocyte or plasma cholinesterases. However, in the majority of studies the blood cholinesterases were the more sensitive. Overall, the Meeting considered that plasma and erythrocyte cholinesterases are adequate surrogates for nervous system acetylcholinesterase after a single oral exposure to phosmet.

In the study in human volunteers (Cameron, 1999), while doses of up to 4 mg/kg bw were given to men, women were given only a dose of 2 mg/kg bw. As no clear effects were seen at the highest dose in each sex, it is not possible to determine whether there is any difference in the susceptibility of men and women to phosmet. In animal studies, there is again no consistent pattern. In some studies, females showed a greater inhibition than males after moderate doses, whereas in other studies this finding was reversed. At doses approximating to NOAELs, the Meeting considered that there was no consistent evidence relating to a sex difference in response to phosmet.

The 1998 JMPR concluded that: ". . . there was no evidence that phosmet could produce clinical signs of delayed polyneuropathy or significantly inhibit neuropathy target esterase" (Annex 1, reference 85).

The 1994 JMPR concluded that there was no evidence for carcinogenicity in either rats or mice (Annex 1, reference 73). The 1998 JMPR concluded that ". . . no further characterization of mutagenicity was required" (Annex 1, reference 85).

In a two-generation study of reproduction in rats given diets containing phosmet (Meyer & Walberg, 1990), reviewed by the 1994 Joint Meeting (Annex 1, reference 73), reductions in mating and fertility were seen at a dietary concentration of ≥80 mg/kg. Reductions of >35% (range, 37–59%) in erythrocyte acetylcholinesterase activity were seen in parental animals at 80 mg/kg. Minimal inhibition (about 10%) of erythrocyte acetylcholinesterase activity was seen in parental animals receiving phosmet at a dietary concentration of 20 mg/kg. Pup weight and survival were reduced at 300 mg/kg. The overall NOAEL was 20 mg/kg (equal to 1.3 mg/kg bw per day) on the basis of parental toxicity and

effects on reproductive performance. The Meeting considered that because the reproductive effects were present at doses that produced significant inhibition of acetylcholinesterase activity and after repeated dosing, a single dose that did not produce significant inhibition of acetylcholinesterase activity would not be likely to produce the reproductive effects.

In study of acute neurotoxicity in rats given phosmet by gavage, erythrocyte acetylcholinesterase activity was inhibited by >70% at 22.5 mg/kg bw and by about 10% at 4.5 mg/kg bw. Brain acetylcholinesterase activity was inhibited by >60% at 22.5 mg/kg bw (Cappon, 1998). These results suggest that rabbits given phosmet at a dose of 15 mg/kg bw (as in the study of developmental toxicity by Moxon, 1991) would show significant inhibition of acetylcholinesterase activity.

Comments

In an acceptable[1] double-blind, randomized study, groups of volunteers received a single dose of phosmet (purity, 96%) or placebo, in a capsule, with water. Six subjects receiving phosmet were paired with three subjects receiving placebo, for each dose. Men received a dose of 1, 2 or 4 mg/kg bw, and women received a dose of 2 mg/kg bw. A wide range of investigations, including assays for erythrocyte cholinesterase activity, was performed before and after dosing (up to 168 h). There were no adverse findings at any dose. The pattern of clinical signs, results of investigations and cholinesterase activities were similar in groups receiving test substance and placebo. The Meeting noted that females had only been given a dose of 2 mg/kg bw and concluded that the overall NOAEL for both sexes was thus 2 mg/kg bw.

The Meeting considered the study in volunteers together with other data on the toxicity of phosmet. The Meeting paid particular attention to the data on fetotoxicity from the study of developmental toxicity in rabbits, which had been used to derive the acute RfD in 1998. The skeletal effects (reduced ossification) seen at 5 mg/kg bw per day in this study were not reproduced at a dose of 15 mg/kg bw per day and were mostly within the ranges for contemporary historical controls for the test facility. The forepaw flexure observed in four out of 132 fetuses receiving a dose of 5 mg/kg bw per day was not present in the database for contemporary historical controls, but there was no dose–response relationship, this finding being present in a single fetus out of 118 receiving a dose of 15 mg/kg bw per day. Taking into account the absence of a dose–response relationship and the data on historical controls, the Meeting concluded that there were no clear compound-related effects at a dose of 5 mg/kg bw per day. The altered ossification observed at 15 mg/kg bw per day was seen in the presence of cholinergic signs and significantly reduced maternal body_weight gain. The Meeting concluded that the fetal effects were unlikely to occur after a single dose that did not induce significant inhibition of acetylcholinesterase activity.

The Meeting established an acute RfD of 0.2 mg/kg bw based on the NOAEL of 2 mg/kg bw (the highest dose tested) for inhibition of erythrocyte cholinesterase in men and women, and a safety factor of 10.

The Meeting recognized that it was possible that the acute RfD might be refined after a full evaluation of the complete database on phosmet.

[1] Annex 5, reference 83, page 5

Dietary risk assessment

International estimated short-term intake (IESTI) for phosmet was calculated for the raw or processed commodities for which appropriate data on residues and consumption were available. The IESTI for the general population represented 0–90% of the acute RfD. The IESTI for children aged ≤6 years represented 0–230% of the acute RfD; the short-term intakes for apples and pears were 150% and 230% of the acute RfD, respectively. The information presented to the Meeting precluded the conclusion that the acute dietary intake for these commodities would be below the acute RfD.

The Meeting concluded that the short-term intake of residues of phosmet from uses that have been considered by the JMPR, with the exception of apples and pears, is unlikely to present a public health concern.

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