DECISIONS REGARDING REQUESTS TO VARY REPORT FOR SECONDARY NOTIFICATION OF CHEMICAL IN OLOA 270

Evaluation of Toxicological Data

1a) Request for variation by Office of The Australian Safety and Compensation Council

Point 1: Variations to the study summaries

In the summaries presented in Section 7.2.6 (Skin sensitisation), the observed incidence of increasing or decreasing skin reactions over the 24 to the 48 hour post-challenge observation period, is reported inconsistently. It would benefit the document to consistently report such observations, and state how long after challenge the reported incidence occurred. Such information can assist in determining whether skin reactions observed at challenge are irritant or allergic in nature. Variations to the study summaries in Section 7.2.6 taking account of the above comments are provided below.

Morris, 1993

Delete '25% of test animals showed skin reactions greater than those seen in control animals'. Replace with the following text:

'The incidence of skin reactions in test animals was seen to increase from 24 to 48 hours post-challenge. At 48 hours, 25 % of test animals showed skin reactions greater than those seen in control animals.'

Kreuzmann, 1993

Delete '25% of test animals showed skin reactions greater than those seen in control animals'. Replace with the following text:

'The incidence of skin reactions in test animals was seen to increase from 24 to 48 hours post-challenge. At 48 hours, 25 % of test animals showed skin reactions greater than those seen in control animals.'

For oedema, it would benefit the study summary to report the number of test animals it was observed in 24 and 48 hours after challenge. Presently the study summary just states, 'Oedema was also noted in 4 of the animals showing grade 2 reactions.'

Morris, 1994a

It is noted that for this study and those by Morris, 1994b; 1996, the sensitisation response in test animals has been determined by subtracting the percentage of control animals giving the same severity of reaction from the percentage of test animals. However, while this approach is clearly the most appropriate for determining the skin sensitisation of the test material in the studies of Morris 1994b; 1996, it is less clear from the summary whether this was the most appropriate for Morris, 1994a.

The Morris (1994a) study summary states, 'All control animals showed grade +/- or 1 skin reactions at 24 and/or 48 hours after challenge. Animals with grade 2 or 3 skin reactions are recorded in the table……10%, 20% and 47% of test animals showed skin reactions greater than those seen in control animals at challenge, rechallenge and cross-challenge, respectively.' It is noted that a grade 2 (or 3?) skin reaction is reported in a single control 48 hours after the initial challenge – it cannot be determined from the summary what the severity was of the skin reaction seen in the control animal.

While +/- skin reactions are considered 'equivocal' and thus excluded when determining the skin sensitisation potential of a substance, it is unclear why grade ≥2 skin reactions in control animals have been included but grade 1 excluded in a 'subtractive' approach. If a subtractive approach is to be used, then grade ≥1 skin reactions in control and test animals should be included. Conversely, if skin reactions in control animals are to be excluded, then only skin reactions in test animals of a higher grade than the maximum observed in control animals are to be used to determine the sensitisation response. Please clarify.

It is requested that the study summary be edited so it is clear to the reader which of the two approaches discussed above has been used in determining the skin sensitisation potential of the test chemical. As it is not clear which approach has been used we are presently unable to submit a reliable variation to the text. Furthermore, it is requested that a statement on whether the incidence of skin reactions were sustained, increased or decreased from 24 to 48 hours post challenge be included in the study summary.

Morris, 1994b

Delete "61% and 50% of test and control animals, respectively, showed grade 1 skin reactions. It was noted that the sensitisation response in test as well as control animals decreased by 48 hours. The overall response (percentage of test animals minus the percentage of control animals) is 11% at 24 hours and 6.7% at 48 hours. A difference in response between test and control animals of 10% does not constitute a positive result.' Replace with the following text:

'The incidence of skin reactions was seen to decrease from 24 to 48 hours post-challenge in both test and control animals. By subtracting from the percentage of test animals from the percentage of control animals with grade 1 reactions at challenge, an overall sensitisation response of 11% and 6.7% was obtained 24 and 48 hours post challenge respectively. A positive result in a Buehler study is an overall skin sensitisation response of 15% or greater.'

Morris, 1996

It is noted that the sensitisation response has been determined by subtracting from the percentage of test animals from the percentage of control animals giving the same severity of reactions at challenge. However, the study summary does not reflect the significant decrease in sensitisation response seen from 24 to 48 hours post challenge.

The borderline sensitisation response seen at 24 hours and its substantial decrease at 48 hours would suggest that the skin reactions seen are likely to be irritant.

Delete 'with 15% more test animals showing grade 1 compared to controls. A difference in response between test and control animals of 15% was obtained at 24 hours post challenge. This is the minimum percentage difference required between the test and control animals for classifying a chemical as a sensitiser. One test animal showed a grade 2 response after 48 hours. Taken together, the finished oil containing 7.6% analogue chemical was considered sensitising to the skin of guinea pigs.' Replace with the following text:

'The incidence of skin reactions was seen to decrease from 24 to 48 hours post-challenge in both test and control animals. By subtracting the percentage of test animals from the percentage of control animals giving the same severity of reactions at challenge, an overall response of 15% was obtained 24 hours post challenge, with a negative response seen at 48 hours. The response seen at 24 hours post challenge is the minimal incidence constituting a positive response in a Buehler study. However, the severe decrease in overall response 24 hours later would suggest that the observed skin reactions in test animals are irritant in nature. Consequently, overall, this study is not considered positive.

The finished oil containing 7.6% analogue chemical was not sensitising to the skin of guinea pigs.'

1b) Decision

Variation approved. The requested deletions and additions have been made to the following studies: Morris 1993; Kreuzmann 1993; Morris 1994b and Morris 1996.

No specific additions/deletions were requested for the Morris 1994a study. The study summary has been edited as follows:

"All control animals showed grade +/- or 1 skin reactions at 24 and/or 48 h after challenge and only one animal showed grade 2 reactions 48 h after challenge. ~~Animals with grade 2 or 3 skin reactions are recorded in the table~~. Animals with skin reactions of a higher grade than the maximum observed in control animals (grade 1) are recorded in the table.

Oedema was also noted in 3 of the animals showing grade 2 reactions at rechallenge, in 8 of the animals showing grade 2 or 3 reactions at cross-challenge and in one test animal showing grade 1 skin reaction at cross-challenge. 10%, 20% and 47% of test animals showed skin reactions greater than those seen in control animals at 24 h after challenge, rechallenge and cross-challenge, respectively. The incidence of skin reactions slightly increased 48 h after challenge (4/20), but was sustained or slightly reduced 48 h following re-challenge or cross-challenge."

Changes to the study summaries do not in any way alter the interpretation of study data or the conclusions from analysing the studies.

2a) Request for variation by Office of The Australian Safety and Compensation Council

Point 2: Histopathological findings in the 90-day repeat dose study.

It is reported in the 90-day repeat dose toxicity study summary (section 7.3.2), that no treatment-related macro- or microscopic changes were seen in females at the end of the recovery period. It would benefit the study summary if a variation is made to also report whether or not histopathological findings were seen at the end of the dosing period. Furthermore, for consistency with the results reported in females, it would also benefit the study summary to report whether the male organ weight changes were seen in the absence/presence of macro- or microscopic changes.

2b) Decision

Variation approved. The following addition has been made to Section 7.3.2 (90-day repeat dose toxicity study); - new text is underlined:

"No corresponding macro- or microscopic findings were observed in the adrenals or liver <u>at the end of the dosing period</u> and no changes were seen <u>in relative organ weights</u> at the end of the recovery period.

Mid and top-dose males showed statistically significant increases in weights of brain (11% and 11%, respectively), liver (11% and 18%, respectively) and kidney (14%, top-dose only) relative to body weight at the 13-week necropsy. Changes were not seen in absolute weights and were considered unlikely to be due to treatment. <u>No histomorphological changes were noted in brain or liver at these doses. Minimal interstitial infiltration of lymphocytes was noted in kidneys in 5/12 rats.</u>

Changes to the study summaries do not in any way alter the interpretation of study data or the conclusions from analysing the studies.

3a) Request for variation by Office of The Australian Safety and Compensation Council

Point 3: Basis for selecting the NOAEL in the 90-day repeat dose study

It is reported in the 90-day repeat dose toxicity study summary (section 7.3.2), that 'general signs of toxicity' were seen in all treatment groups in a dose-dependent manner i.e. at 100, 500 and 1000 mg/kg bw/day. However, a NOAEL of 100 mg/kg bw/day was identified based on a statistically significant increase in relative adrenal weight in females. Consequently, it would benefit the study summary if the following variation is made to the last paragraph.

'The minimal signs of clinical toxicity observed in animals at 100 mg/kg bw/day were seen in the absence of any other signs of toxicity, and consequently are not considered biologically significant. A No Observed Adverse Effect Level of 100 mg/kg bw/day was identified in females based on clinical signs of toxicity together with a statistically significant increase in relative adrenal weight (Chengelis et al., 1995).'

Additionally, if the clinical signs of toxicity seen at 100 mg/kg bw/day were only seen in a small number of animals (the incidence is not reported in the study summary) then this should also be included in the above rationale of why 100 mg/kg bw/day was not considered a LOAEL.

3b) Decision

Variation approved. The requested variation has been made to the 90-day repeat dose toxicity study (Section 7.3.2).

4a) Request for variation by Office of The Australian Safety and Compensation Council

Point 4: Variation to study summaries

In section 8.2.1 (Skin sensitisation), information on skin reactions seen during induction are reported under 'Challenge outcome' in the study summaries for Harper et al., 1995 and Buehler et al., 1993b. It would be more appropriate to report induction findings separately from challenge results. Therefore, the following variations are proposed.

For Harper et al., 1995 delete 'One subject showed a reaction at the induction site immediately before the challenge application was made.

Fourteen days after the end of the induction phase, the test material was applied to previously untreated sites, under semi-occlusive dressing for 24 h. A confirmatory rechallenge was conducted in the subject, approximately 7 weeks after the primary challenge.

Challenge outcome: This subject also showed sensitisation reactions 96 h after the primary challenge and 96 h after challenge.

Two subjects exhibited mild erythema at the second and eighth induction visit, respectively. The responses resolved within the following two visits.' Replace with the text below, following on from the end of the first paragraph:

'Fourteen days after the end of the induction phase, the test material was applied to previously untreated sites, under semi-occlusive dressing for 24 hours.

Two subjects exhibited mild erythema at the second and eighth induction visit, respectively. The responses resolved within the following two visits. One subject showed a skin reaction at the induction site immediately before the challenge application was made.

Challenge outcome: A sensitisation reaction was seen 96 hours after challenge in the subject showing a skin reaction immediately before the challenge application. A confirmatory rechallenge was conducted in this subject 7 weeks after the initial challenge. A sensitisation reaction was again seen, 96 hours after rechallenge.'

Similarly, for Buehler et al., 1993b delete ‘Approximately 2 weeks after the end of the induction phase, test chemical was applied to previously untreated sites, under semi-occlusive dressing for 24 h.

Challenge outcome: There was no evidence of sensitisation reactions in any subjects following challenge with either test chemical.

Responses to both test oils were generally mild, with transient mild erythema being observed during induction and challenge phases.’ Replace with the text below, following on from the end of the first paragraph:

‘Approximately 2 weeks after the end of the induction phase, the test chemical was applied to previously untreated sites, under semi-occlusive dressing for 24 hours.

Responses to both test oils were generally mild, with only transient mild erythema being observed during induction.

Challenge outcome: Responses to both test oils were generally mild, with only transient mild erythema being observed during challenge. Consequently, this study provides no robust evidence of a sensitisation reaction in any subject following challenge with the test material in either oil.’

4b) Decision

Variation approved. The requested deletions and additions have been made to the following studies: Harper et al. 1995 and Buehler et al. 1993b. Changes to the study summaries do not in any way alter the interpretation of study data or the conclusions from the analyses of the studies.

5a) Request for variation by Office of The Australian Safety and Compensation Council

Point 5: Variation to Study summary

In section 8.2.1 (Skin sensitisation) it is stated in the Boisits et al., 1993 study summary that for skin reactions seen during induction ‘Sixteen of these were considered to be sensitisation reactions’. However, the summary also contains the statement ‘In the absence of a challenge phase, no reliable conclusions can be drawn on the skin sensitisation potential of the test material from the study’. Therefore, is the statement on the sixteen sensitisation reactions from the study authors or the NICNAS assessor? If it is from the NICNAS assessor, then the summary is conflicting and requires editing. If the statement is from the authors of Boisits et al., 1993, then the following variation is proposed.

Delete ‘Nineteen individuals experienced intense reactions either during the course or on completion of the induction phase of the study. Sixteen of these were considered to be sensitisation reactions. Subjects with severe reactions needed medical treatment to help relieve symptoms taking up to 6 weeks in the 3 most severe cases. Due to

intensity of skin reactions produced during induction, the challenge phase of the study was not conducted.

In the absence of a challenge phase, no reliable conclusions can be drawn on the skin sensitisation potential of the test material from this study.' Replace with the following text:

'Nineteen individuals experienced intense reactions either during the course or on completion of the induction phase of the study. Subjects with severe reactions needed medical treatment to help relieve symptoms taking up to 6 weeks in the 3 most severe cases. Due to intensity of skin reactions produced during induction, the challenge phase of the study was not conducted.

The study authors report that intense skin reactions seen in sixteen individuals during induction were considered to be sensitisation reactions. However, in the absence of a challenge phase, no reliable conclusions can be drawn from this study on the skin sensitisation potential of the test material.'

5b) Decision

Variation approved. The requested deletions and additions have been made to the Boisits et al. 1993 study. Changes to the study summary do not in any way alter the interpretation of study data or the conclusions from the analysis of the study.