Appendix B: Toxicity of 1080

Contents

List of tables

Key points

Human health classifications (class 6) for 1080 technical grade

Table B1: Human health classifications (class 6) for 1080 technical grade

	Applicant and Agency
1080 technical grade active	Toxicity: 6.1A, 6.3B, 6.4A, 6.8A, 6.9A

- The Agency agrees with the applicants' classifications for technical grade 1080.
- The high acute toxicity of 1080 is well known.
- 1080 can also cause reproductive toxicity in male mammalian species and developmental effects in laboratory rodents.
- 1080 can cause sub-chronic toxicity affecting the heart after prolonged exposure in laboratory rodents and, possibly, in sheep.

Human health classification (class 6) for 1080 formulations

- The Agency agrees with the applicants' classifications for 1080 formulations.
- There is one change in the Agency's classification for a formulated product from that applied by the Hazardous Substances (Sodium Fluoroacetate) Transfer Notice 2005. "Gel containing 1.5 g/kg sodium fluoroacetate" was classified as a contact sensitiser (6.5B) in that notice on the basis of a component present in the formulation at low concentration with this hazard. The Agency recommends this classification is removed.

Data quality and completeness

- Despite efforts over the past decade to address data gaps in the toxicology database for 1080, deficiencies remain in the information available. However, the information the Agency has reviewed is considered sufficient to assign classifications to 1080 and substances containing 1080.
- Some studies have not been carried out in accordance with modern international test guidelines. The Agency did not consider that this introduced significant uncertainty to any of the critical findings.
- The Agency considers that a multi-generation reproductive study is desirable to clarify the extent of the reproductive toxicity hazard. However, such a study is not considered essential to this assessment as effects have been assumed to be serious and relevant to human exposures.
- Other data on 1080 that would be desirable, but which are also not considered essential for the assessment, are studies on dermal absorption, acute inhalation, and studies on the reversibility of the effects on the male reproductive system.

- The lack of any chronic toxicity/carcinogenicity study is not considered by the Agency to be essential because of the negative mutagenicity findings (class 6.6), and the unlikelihood of chronic exposure to the substance.
- The lack of data for a multi-generation study and for a chronic toxicity/carcinogenicity study results in the use of additional uncertainty factors in the derivation of the AOEL and ADE values for the human risk assessment in Appendix M. This means that a more conservative approach has been taken to the assessment of health risks.

Human toxicity of 1080

Human poisonings indicate that with respect to acute toxicity of 1080, effects on humans are similar to other terrestrial mammalian species, so the animal model data are relevant to the assessment of human health risks.

Antidotes and treatment for 1080

Specific antidotes are not available for treatment of acute poisoning from 1080; nevertheless therapeutic intervention may be effective in achieving recovery after serious toxic effects from 1080 intake.

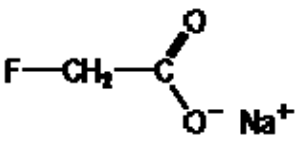
B1 Toxicity classification summary for technical grade 1080 (the active ingredient)

B2 General introduction

The Agency has assessed the information supplied by the applicants, together with that available from other sources, and assigned class 6 toxicity classifications to technical grade 1080.

Information relating to the identification of 1080, sodium fluoroacetate, technical grade active is provided in Table B2.

Table B2: Identification of 1080 technical grade active

Identification of the substance:		
This summary is compiled from both confidential and non-confidential information supplied by the applicants.		
Annex I index No. -	**EC No. -**	**CAS-No: 62-74-8**
IUPAC name	Fluoroacetic acid, sodium salt	
Common names	Sodium monofluoroacetate, Compound 1080, 1080	
Molecular formula	$C_2H_2FO_2.Na$	
Structural formula		
Molecular weight	100.03	
Purity (wt/wt)	90% (w/w) minimum (typically 95–98.5% in analysis by importer)	

Identification of the substance:	
Significant impurities or additives, their concentrations (wt/wt)	Potassium fluoride, 1% maximum Ethyl chloroacetate, 1% maximum Sodium hydroxide, 0.5% maximum Methanol, 0.5% maximum Water, 6.9% maximum
Known uses	Vertebrate Toxic Agent and Insecticide (for wasps)
HSNO classification (toxicity)	6.1A, 6.3B, 6.4A, 6.8A, 6.9A
Other classification & labelling	UN No. 2629

B3 Acute oral toxicity (6.1 classification)

B3.1 Introduction

The Agency has reviewed the information provided in the application, including the associated references and information from other sources.

1080 was found to be extremely toxic in the mid 1940s when its use as a rodenticide and vertebrate poison first commenced (Chenoweth 1949). Acute exposure to 1080 is followed initially by a latency period of typically 30 minutes – 2 hours, without any signs or symptoms. If a toxic dose has been received, the latency period often ends abruptly with the development of serious toxic effects in the exposed animal. While there are differences between species, the major toxic effects are cardiac disorders, including changes in heart rhythm (arrhythmia), which may progress to ventricular fibrillation, and may often be fatal. In other cases death is due to heart failure. The most striking toxic effects, when they occur, are seizures. There appears to be some variation in species susceptibility to seizures from 1080. Seizures commonly cause oxygen deprivation (hypoxia) if prolonged and may be fatal due to respiratory arrest. The mechanism of toxicity is discussed separately in section B16.

B3.2 Results

A range of acute oral toxicity (LD_{50}) values are available for 1080 in laboratory and terrestrial vertebrates. Table B3 lists the oral LD_{50} values for 1080 in laboratory animal species. Values for large farm mammals and wild species of relevance to the ecotoxicity of 1080 to terrestrial vertebrates are not listed here (see Appendix C).

Table B3: Acute oral LD_{50} values for 1080 in laboratory mammalian species

Species	Sex	Value (mg/kg bw)	Date	Reference
Rodents				
Rats, Albino (Strain unspecified)	U[1]	2.5	1949	Chenoweth, 1949 (cited reference 74)
Rat, Norway (Wild adult, Maryland)	U	0.22	1949	Chenoweth, 1949 (cited reference 44)
Rat, Norway (Florida)	U	3.0	1949	Chenoweth, 1949 (cited reference 129)

Species	Sex	Value (mg/kg bw)	Date	Reference
Rat (Strain unspecified)	U	0.1	1946	Fairchild et al, 1977 (cited American JPH and the Nation's Health, Vol 36, 1427, 1946)
Rat, Norway	Male Female	2.1 2.2	1971	Atzert, 1971 (cited Denver Wildlife Centre, unpublished)
Alexandrine rat (*Rattus rattus alexandricus*)	U	0.5	1949	Chenoweth, 1949
Black rat (*Rattus rattus*)	U	0.1	1949	Chenoweth, 1949
House mouse (*Mus musculus*)	U	8.0	1949	Chenoweth, 1949
Deer mouse (*Peromyscus* spp.)	U	4.0	1949	Chenoweth, 1949
Mouse, Albino (Carworth)	U	17.0	1949	Chenoweth, 1949
Mouse (Strain unspecified)	U	0.1	1977	Fairchild et al, 1977 (Cites YKHUA6. Yukkyoku. Pharmacy, Tokyo. Vol 28, p329, 1977
Guinea pig (Strain unspecified)	U	0.3	1946	Fairchild et al, 1977 (Cites American JPH and the Nation's Health, Vol 36, p1427, 1946.
Langomorphs				
Black-tailed jack rabbit (*Lepus californicus*)	Male	5.55	1971	Atzert, 1971 (cites Denver Wildlife Centre, unpublished)
Rabbit (Strain unspecified)	U	1.0	1969	Yashimoto et al, 1969
Rabbit (Strain unspecified)	U	0.34	1982	Fairchild et al, 1977 (cites AWLRAO Australian Wildlife Research Vol 9, p487, 1982)
Small mammals				
Oral cat (Species unspecified)	U	0.35	1948	Fairchild et al, 1977 (cites J Amer. Pharmaceutical Association, Vol 37, p307, 1948)
Dog (Species unspecified)	Male	0.066[2]	1950	Tourtellotte and Coon, 1950
Primates				
Monkey[3, 4] (Species unspecified)	U	300	1969	Yashimoto et al, 1969
Monkey[3] (Species unspecified)	U	4	1978	Rammell and Flemming, 1978

Notes

1 U means unreported.

2 This is the lowest oral LD50 value and is used for classification of 1080 and mixtures containing it.

3 These values are considered unreliable, see text.

4 The journal article indicates that the value is a minimum lethal dose.

B3.3 Data quality

The Agency usually requires results based on studies carried out in accordance with the requirements of Good Laboratory Practice (GLP) and an appropriate test guideline such as the OECD Chemical Test Guidelines. The data for 1080 are relatively old and most, if not all, of the acute toxicity studies were performed before such guidelines had been developed. However, the Agency does not consider this to represent a significant deficiency in the information overall because there is quite a lot of information available and the findings are consistent.

The Agency does not consider that carrying out further acute toxicity studies using the oral route in accordance with guidelines is justified. Rather than carrying out acute tests (using new acute guideline methods, OECD Guideline Nos 422 and 423 (which replaced the superseded OECD Guideline No 401 for the oral LD_{50} test), it would be preferable to do more targeted tests to compare the sensitivity of species and strains to resolve data anomalies on a case-by-case basis.

The Agency does not consider such studies are essential before a decision can be reached on this application.

Another aspect relating to data quality, relates to the accuracy of some reported values. The oral LD_{50} value in dogs is often reported as 0.06 mg/kg bw. Indeed this is the value cited by the applicants. The Agency examined the reference cited by the applicant (Chenoweth 1949) for this value and it appears incorrect. The original paper (Chenoweth and Gilman 1946), which is cited by Chenoweth (1949), only reported data from intravenous exposure in dogs, and reported the intravenous LD_{50} in dogs as 0.06 mg/kg bw. A further confusing aspect is that this value actually was the finding from intravenous administration of methyl fluoroacetate (not 1080, sodium fluoroacetate) in dogs. Chenoweth and Gilman (1946) explained why methyl fluoroacetate was used and this is discussed in B6.3.

B3.4 Discussion of acute oral toxicity

The data for primates are very sparse. The reported LD_{50} value in monkeys, 300 mg/kg bw (Fairchild et al, 1977), appears unreliable. A footnote in the original source indicates this value is a minimum lethal dose not an LD_{50} value (Yashimoto et al, 1969) (The article is discussed further under acute dermal toxicity in section B4.) Rammell and Fleming (1978) did not provide a specific source for their LD_{50} of 4 mg/kg bw listed for monkey. It may have been sourced from Atzert (1971) and listed incorrectly as an oral LD_{50} value. On the basis of these two values, Rammell and Fleming suggest that primates are relatively less sensitive to 1080 than other species. The Agency considers these data too unreliable to indicate whether or not primates are less sensitive to 1080 than other species, in general, but consider it well established that dogs, and related carnivores, are more sensitive than other terrestrial vertebrates including humans.

There are some data relating to human poisoning cases. Although these data are of assistance in regard to expert judgement and human risk assessment, they cannot be used directly for classification of 1080. This information is discussed in detail in section B17.

B3.5 Conclusion

The classification for acute toxicity 6.1 is usually based on the lowest oral LD_{50} value in a suitable laboratory mammalian species. The most appropriate value on which to base classification of 1080 is the dog LD_{50} value of 0.066 mg/kg bw (Tourtellotte and Coon 1950). The Agency concluded this value should be used for classification of 1080 for acute oral toxicity. The Agency has not to rounded the value to 0.07 mg/kg bw, although others appear to have done so (Rammell and Fleming 1978).

This value gives 1080 a classification of 6.1A according to the Hazardous Substances (Classification) Regulations 2001.

- ***Classification of 1080: Acute oral toxicity: 6.1A***

B4 Acute dermal toxicity (6.1)

B4.1 Results

The data available for the dermal toxicity of 1080 are listed in Table B4.

Table B4: Dermal LD_{50} values

Species	Sex[1]	Value (mg/kg bw)	Date	Reference
Mouse	U	25.3	1969	Yashimoto et al, 1969
Rat	U	48	1969	Yashimoto et al, 1969
Guinea pig	U	1.6	1969	Yashimoto et al, 1969
Rabbit	U	1.5	1969	Yashimoto et al, 1969
Rabbit	Male	277	1977	Fagerstone, et al 1994
	Female	324		(Cites Savarie and Cerven, unpublished)
Rabbit	U	1.5	1969	Fairchild et al, 1977 (Cites Yashimoto et al, 1969
Rabbit	Male	277	1995	USEPA, RED, 1995
	Female	324		

Note:

1 U means unreported.

B4.2 Data quality

Dermal LD_{50} data are limited. However, one study has been carried out in accordance with United States Environmental Protection Agency (USEPA) Guideline 81-2.

Comparative studies too would be useful to determine the absorption rates according to more up-to-date methodology. This would also enable inter-species comparisons of absorption of 1080. The Agency notes that at least some stages of such tests can be done using *in vitro* techniques and this

enables estimates of dermal absorption rate for humans to be made. While this information would be useful, the Agency does not consider such studies are essential before a decision can be reached on this application.

B4.3 Discussion

Fagerstone et al (1994) reported the dermal LD_{50} of technical grade 1080 in male rabbits as 277 mg/kg bw based on an unpublished report (Savarie and Cerven, unpublished). The animals showed lethargy, diarrhoea and convulsions preceding death, along with extensive haemorrhage of the thymus and congestion of the lungs. The study author indicated the study was performed in compliance with USEPA Guideline 81-2. The Agency notes these data were used by the USEPA to assign 1080 to Category II as a dermal toxicant (USEPA RED, September, 1995).

The only other available data indicated much higher dermal toxicity for 1080 in four mammalian species including rabbits (Yashimoto et al, 1969) The rabbit dermal toxicity value from this study is nearly two orders of magnitude higher than the Savarie and Cerven result. Although the dermal toxicity results from this report were cited by another US government source (Fairchild et al, 1977), they were not referenced or taken into account in the US RED, so the Agency did not consider them authoritative (USEPA RED, September, 1995).

B4.4 Conclusion

While the Agency usually uses the lowest LD_{50} value to derive classifications, the data used by the USEPA appear to be the most reliable (as it conforms to international guidelines). The dermal LD_{50} of technical grade 1080 in male rabbits as 277 mg/kg bw was used as the basis for the dermal classification of 1080. This gives 1080 a classification of 6.1C according to the Hazardous Substances (Classification) Regulations 2001.

- ***Classification of 1080: Acute dermal toxicity: 6.1C***

B5 Acute inhalation toxicity (6.1)

B5.1 Introduction

The Agency found there are no inhalation LC_{50} values available that can be used for classification of 1080.

B5.2 Data quality

The lack of acute toxicity data for 1080 represents a data gap. It seems to be generally agreed nevertheless, that 1080 is of high acute toxicity by inhalation. Therefore, the Agency concludes that rather than in determining the LC_{50} values to verify this, it would be more appropriate to investigate of inhalation absorption rates from exposures to different forms (dust and mist) and this work is likely to verify the assumption of high inhalation toxicity for 1080.

The Agency does not consider such studies are essential before a decision can be reached on this application.

B5.3 Discussion

One case of human exposure to 1080 in the form of a dust indicated that 1080 is likely to be highly toxic to humans by inhalation. The first hand account of a single inhalation exposure to 1080 in the form of powder was reported by the patient (Williams 1948). Severe symptoms, including unconsciousness, occurred. While the dose received was uncertain, the indication was of a relatively low dose, referred to as "a single puff of the powder". In view of the serious toxicity that resulted, this suggested 1080 is highly toxic by inhalation in humans.

The Hazardous Substances (Classification) Regulations 2001 (Schedule 4) do not provide for the assignment of a 6.1A (inhalation) classification other than on the basis of an appropriate LC_{50} result. Only in the case of the 6.1E (inhalation) does Schedule 4 allow, in clause (b), for the use of information other than a measured LC_{50} value, such as human data or expert judgement for classification. If the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001 have been triggered by clause 2(d) or clause 2(e) the only classification that can be assigned under the Hazardous Substances (Classification) Regulations is 6.1E. Therefore, the human exposure report could only be used as a basis for classification of 1080 as 6.1E (inhalation).

B5.4 Conclusion

The expert view held by the Agency and other competent bodies (American Conference of Governmental Industrial Hygienists (ACGIH), 1991), is that the 1080 dust or mist is highly toxic, if inhaled. Therefore, although the Agency found the available data are "Insufficient data to classify 1080 for inhalation toxicity", it has proposed in section 8.2.2 that section 77A of the HSNO Act should be used to assign controls to 1080 equivalent to those that would apply if it were classified 6.1A for inhalation.

- ***Classification of 1080: Inhalation toxicity: Insufficient data (no classification)***

B6 Acute toxicity (other) (6.1)

B6.1 Introduction

This relates to acute toxicity by a route other than oral, dermal or inhalation. In practice this means exposure by injection.

B6.2 Results

The data available for the toxicity of 1080 by intravenous injection are listed in Table B5.

Table B5: LD_{50} values for 1080/methyl fluoroacetate[1] by intravenous injection in some laboratory species[2]

Species	Value (mg/kg bw)	Date	Reference
Dog (*Canis familiaris*)	0.06[2, 3]	1949	Chenoweth and Gilman, 1946
Cat (*Felix domesticus*)	0.2		Chenoweth, 1949 (cited Modell et al, undated private communication)
Rabbit (stated to be "several varieties")	0.20–25	1949	Chenoweth and Gilman, 1946
Rhesus monkey (*Macaca mulatta)*	4.0	1949	Chenoweth and Gilman, 1946
Spider monkey (*Ateles geoffroyi*)	14.0[4]	1949	Chenoweth and Gilman, 1946
Monkey (species not specified)	5.0	1946	Lewis, 1992 (cites (Cites American JPH and the Nation's Health, Vol 36, 1427, 1946)

Notes

1 The sex of the animals was not given.

2 The original source (Chenoweth and Gilman 1946) indicates these values apply to methyl fluoroacetate. The relevance of these data to 1080 is discussed in the text.

3 Cited by applicant as an oral value.

4 Cited by Chenoweth (1949) as 15 mg/kg bw.

B6.3 Discussion

The values are cited by Chenoweth (1949) for 1080, but according to the source paper (Chenoweth and Gilman 1946) are values for methyl fluoroacetate. The authors explain that 1080 and methyl fluoroacetate behave similarly from a pharmacological perspective, and methyl fluoroacetate was used as it could be readily purified by distillation (while 1080 could be contaminated with sodium fluoride at that time). It is not surprising that the methyl ester would be readily hydrolysed, so that little difference would be found between responses to the substances, particularly after injection. The minor correction for the molecular weights of the compounds, 1080 (MW = 100) in comparison to methyl fluoroacetate (MW = 92), is so small as to be insignificant, when other influences the LD_{50} toxicity parameter (such temperature) are taken into account (Oliver and King 1983).

The data from Chenoweth and Gilman (1946) is often quoted as applying to 1080 without comment on the compound actually used in the study. As noted in section B3, the LD_{50} value listed for dogs (0.06 mg/kg bw), has been cited by other sources, including the applicant, as an oral value for 1080 rather than an intravenous value for methyl fluoroacetate (Chenoweth and Gilman 1946). It may be this was due to the observation by the study author that the values for oral and injection routes were virtually identical.

B6.4 Conclusion

No classification provided for under the Hazardous Substances (Classification) Regulations 2001 relating to the injection route.

Therefore, the Agency did not assign a 6.1 classification for injection as there is no provision for this under the HSNO Act.

In view of the observation of the similarity of toxicity of 1080 by oral and injection routes the Agency documented the injection data. The similarity of the oral and injection LD_{50} values supports that view that 1080 is readily absorbed as discussed in section B15.

B7 Skin irritancy (6.3)

B7.1 Introduction

No studies assessing the skin irritancy of technical grade 1080 were identified by the Agency.

B7.2 Data quality

A dermal irritation study for 1% 1080 complying with USEPA guideline 81-5 has been submitted to the USEPA.

B7.3 Results

Fagerstone et al (1994) cited an unpublished skin irritation study (Savarie and Cerven, unpublished report). Six albino rabbits were treated dermally with 1% 1080 and the substance kept on the skin for four hours. There was not erythema (redness), but slight oedema (swelling) was found in some animals up to 5 hours after treatment. There were no effects at latter observation periods. The study was cited in the USEPA RED (USEPA, 1995) with the following citation (Cerven 1987a).

B7.4 Conclusion

The Agency concluded a 6.3B classification is appropriate for the technical grade 1080, on the basis that a 1% solution produced slight irritation in this rabbit study.

- ***Classification for 1080: Skin irritancy: 6.3B***

B8 Eye irritancy (6.4)

B8.1 Introduction

No studies assessing the eye irritancy of technical grade 1080 were identified by the Agency.

B8.2 Data quality

An eye irritation study for 1% 1080 complying with USEPA guideline 81-4 has been submitted to the USEPA.

B8.3 Results

Fagerstone et al (1994) cited an unpublished eye irritation study (Savarie and Cerven, unpublished) in rabbits using 1% technical 1080. No corneal opacity or iritis was observed, but there was slight conjunctival irritation and slight chemosis.

The study was also cited in the USEPA RED (USEPA, 1995) and cited as (Cerven 1987b).

B8.4 Conclusion

The Agency concluded a 6.4A classification is appropriate for the technical grade 1080, on the basis of the mild irritation reported for a 1% aqueous solution in rabbits.

- ***Classification for 1080: Eye irritancy: 6.4A***

B9 Respiratory sensitisation (6.5A)

B9.1 Lack of data

The Agency was unable to locate any studies on the ability of 1080 to cause respiratory sensitisation. The Agency did consider such studies are essential before a decision can be reached on this application.

B9.2 Conclusion

The Agency, therefore, found no evidence of respiratory sensitisation for 1080. There was insufficient data to assign a classification on the end point is not triggered.

- ***Classification for 1080: Respiratory sensitiser (6.5A): Insufficient data (no classification)***

B10 Contact sensitisation (6.5B)

B10.1 Lack of data

The Agency was unable to locate any studies on the ability of 1080 to cause contact sensitisation. The Agency did consider such studies are essential before a decision can be reached on this application.

B10.2 Conclusion

The Agency, therefore, found no evidence of contact sensitisation for 1080. There was insufficient data to assign a classification on the end point is not triggered.

- ***Classification for 1080: Contact sensitiser (6.5B): Insufficient data (no classification)***

B11 Mutagenicity (6.6B)

B11.1 Data quality

The Agency was unable to verify that the mutagenicity studies reported below were carried out in accordance with an established international guideline. However, the studies were performed relatively recently and the reports indicate compliance with the research companies protocols. The Agency notes that the findings are cited by the USEPA RED (USEPA, 1995).

B11.2 In vitro studies

An Ames assay, using histidine reversion in *Salmonella typhimurium* (TA1535, TA1437, TA98 and TA100) and *Escherichia coli* (WP2 *uvr*A) with and without metabolic activation (s9) to detect point and frame shift mutations was carried out with 1080 at 10, 31.6, 100, 316 and 1000 μg/plate. No mutagenicity was observed in the presence or absence of metabolic activation (MPI Research 1998a).

A mouse lymphoma assay was carried out using mouse lymphoma (L5178Y) cell line cells *in vitro* with the thymidine kinase gene as the endpoint to detect mutations. This test detects mammalian gene mutations (*in vitro*). 1080 was tested at 5 to 5000 μg/ml. No mutagenicity was observed (MPI Research 1998b).

B11.3 In vivo studies

A mouse micronucleus test, was carried out Swiss Webster mice (males and females) were dosed orally at 0.75, 1.5, 3.0, 6.0 and 7.5 mg/kg bw. No increase in micronuclei was observed in bone marrow extracts. Lethality and toxic effects were observed in the two top dose groups. This was consistent with the high oral toxicity of 1080 as reported in section B3 (MPI Research 1998c).

B11.4 Conclusion

The results of these three complementary genotoxicity studies were reported to provide strong support for the hypothesis that 1080 is not genotoxic (Eason et al 1999). The Agency notes that the final reports do not refer to a study design according to the relevant OECD Guidelines 474, 476 and 474, respectively, but Appendix A: Protocol for each of these studies, which was not available to the Agency.

The Agency's *User Guide to HSNO Thresholds and Classifications* indicates that only the *in vivo* test systems provide a definitive basis for HSNO Act classification. The Agency considers that the negative finding in the *in vivo* study provides sufficient information to conclude that 1080 does not trigger mutagenicity.

- ***Classification for 1080: Mutagenicity: 6.6 is not triggered***

B12 Carcinogenicity (6.7)

B12.1 Lack of data

The Agency did not find any studies that had been carried out to determine whether or not 1080 can case carcinogenicity in any mammalian species. No long-term (life-time) toxicity or carcinogenicity bioassays using 1080 have been carried out.

While the mutagenicity data are negative, carcinogenicity data are usually required for substances for which chronic exposure in humans is likely. In the case of 1080, even considering its extensive proposed use, the likelihood of prolonged exposure, for example, by exposure to residues in food, as may occur for other pesticides, is very unlikely. Regular prolonged exposure to 1080 is highly unlikely even for workers as exposures are intermittent. Therefore, the Agency did not consider such studies are essential before a decision can be reached on this application.

B12.2 Conclusion

The Agency notes that the mutagenicity data for 1080 are negative. This indicates there is no reason to suspect that 1080 may be carcinogenic.

- ***Classification of 1080: Carcinogenicity (6.7): Insufficient data (no classification)***

B13 Developmental and reproductive toxicity (6.8)

B13.1 Introduction

The Agency has reviewed data related to this classification in two separate areas:

- developmental (teratology) studies
- reproductive toxicity studies

B13.2 Developmental (teratology) studies

B13.2.1 Data quality

The data for developmental effects (teratology) are relatively sparse. Detailed studies carried out in recent decades in accordance with appropriate test guidelines have only been carried out in rats. Studies in other species commonly used for regulatory decision-making, particularly rabbits are not available.

Further studies in other species, particularly rabbits, are desirable, as species variability in relation to developmental toxicity is well recognised. Investigations of the mechanisms by which 1080 causes these effects are also desirable, as discussed further in section B16.

The Agency did not consider that the lack of these studies was crucial or prevented decision making on this application.

B13.2.2 Results and discussion

Teratology study in Sprague-Dawley rats (MPI Research/Landcare 1988)

Study summary

Eason et al (1999) cites the results for a teratology study in summarised form. The only reference provided (in Table 3 of the paper) is MPI Research/Landcare Research 1998. The Agency does not have a copy of the study report and assumes it is unpublished. The authors of the journal article provide a summary, and since this is the definite study for this endpoint it is reproduced with minor changes to aid clarity in brackets below.

> A total of 26 female Sprague Dawley rats per treatment group were dosed orally with 1080 solution (concentration not specified) at 0.0 [water only] 0.1, 0.33 or 0.75 mg/kg bw/day from day 6 - 17 of gestation and euthanised on day 20.
>
> No maternal or clinical toxicity was observed at any dose. However, decreased maternal body weight, weight gain and food consumption were observed at 0.75 mg/kg/day during dosing (so this was considered to be the maternal [lowest observed adverse effect level (LOAEL)]). This was accompanied by decreased foetal body weight at 0.75 mg/kg bw/day.
>
> As is standard practice in these studies, half the foetuses were fixed for examination of soft tissue. No external or visceral (soft tissue) abnormalities were observed at any dose. The remaining (half) foetuses were fixed for skeletal examination. Treatment-related skeletal abnormalities were observed at 0.33 and 0.75 mg/kg bw/day. Abnormal development of the forelimb, characterised by bent scapula, humerus, and radius or ulna, was observed in 24%, 12% and 8% percent of litters, respectively at 0.75 mg/kg bw/day (for these abnormalities). The changes were mild but treatment related and classed as malformations ie irreversible alterations of skeletal development.
>
> Bent ribs were observed in 20% and 52% of litters at 0.33 and 0.75 mg/kg bw/day respectively (for these types of abnormality). Unossified sternebrae were also observed in 72% of litters at 0.75 mg/kg bw/day. These developmental abnormalities are classed (by the authors) as variations as opposed to malformations because they are considered to be reversible, and may potentially be due to maternal stress rather than a direct effect of the toxin on the foetus.

Agency review

The Agency notes that the pilot study referred to lower litter size at the top dose level (1.0 mg/kg bw/day). The Agency found the article unclear on this point, however, as there seemed to be an inconsistency between this statement and the statistical parameters reported. If there were no effects observed on uterine parameters (gravid weight, number of implantations, resorptions, live and dead foetuses) at any dose level, it is difficult to see how litter size could be lower at 1 mg/kg bw. Since the Agency did not have access to the full research report, this could not be clarified.

In relation to the main study, the no observed adverse effect level (NOAEL) for maternal toxicity was determined to be 0.33 mg/kg bw/day, based on effects on maternal body weight at 0.75 mg/kg bw/day (based on decreased body weight and body weight gain and food consumption). There is no statement made in the summary relating to the uterine parameters, but the numbers listed in Table 2 (of Eason et al 1999) indicate a slight reduction in litter size with increasing dose. The numbers of foetuses being 160, 160, 156 and 151 in the 25 litters at the dose levels 0, 0.1, 0.33 and 0.75 mg/kg bw/day respectively. The Agency notes this reduction is small and occurs at dose levels that produce maternal toxicity. Since no comment is made on this by Eason et al (1999) it is assumed this difference was not statistically significant.

The NOAEL for developmental effects was considered by the researchers to be 0.1 mg/kg/day, based on the finding of a dose-related increase in forelimb abnormalities at 0.33 mg/kg bw/day and above. Other forelimb abnormalities were found at the higher dose level (0.75 mg/kg bw/day). Bent ribs observed at 0.33 mg/kg bw/day and ossification defects in sternebrae found at the higher dose level (0.75 mg/kg bw/day) were not considered by the researchers to be developmental abnormalities.

Study outcome

Since the developmental effects in offspring are seen at dose levels which are not associated with toxic effects in the maternal animals, the abnormalities are not considered to be caused by secondary effects (the result of toxic effects in adult female animals). This makes the findings of greater toxicological significance.

<u>Maternal</u>

NOAEL = 0.33 mg/kg bw/day

LOAEL = 0.75 mg/kg bw/day

(based on decreased body weight and body weight gain and food consumption)

<u>Foetal</u>

NOAEL = 0.1 mg/kg bw/day

LOAEL = 0.33 mg/kg bw/day

(based on the finding of a dose-related increase in forelimb abnormalities)

Other developmental (teratology) findings

There are a number of other reports relating to reproductive and developmental toxicity findings for 1080. Although these are not suitable in themselves for classification, they provide supporting evidence and established the need for subsequent investigations. The early studies investigated developmental toxicity and the intent of these studies was to investigate teratogenicity mechanisms. A brief summary of these findings is provided below.

De Meyer et al (1964)

The first study used *in vitro* techniques to investigate the metabolic mechanisms that may be responsible for teratogenicity of 1080 in rats (De Meyer et al 1964). There is a reference in the introduction to 600 mg /kg bw dose of 1080 by intra peritoneal injection on the 9th day causing eye anomalies, syndactylia and evisceration. This appears to an unreferenced statement relating an earlier study. The information is incomplete. The Agency notes that recent authors also refer to the lack of information in the report (Eason et al 1999). The *in vitro* methodology of the type employed by the study is not suitable for regulatory purposes, and no findings are of relevance.

Spielman et al (1973)

In a subsequent investigation Spielman et al (1973) reported that they could not confirm teratology "findings" that they attributed to De Meyer et al (1964). As noted above, the Agency does not consider the earlier findings attributed to De Meyer et al (1964) were adequately documented. In this investigation, a dose of 1 mg/kg bw was administered on each of days 8, 9, 10 and 11 to pregnant Wistar rats, or a single 1 mg/kg bw dose was administered on day 9, 10 or 11. At least 40 embryos per dose from these animals were examined at day 20. No visible malformations were found. There was no difference in weights of embryos or placentas. Skeletal staining also revealed no abnormalities.

Agency discussion

The negative findings in this study are not supportive of more recent investigations (Eason et al 1999), in which skeletal abnormalities in rats exposed to 1080 were demonstrated. One explanation for the difference may be the difference in rat strain, Spielman et al 1973 used Wistar rats while Eason et al (1999) used Sprague-Dawley rats, but the Agency was not aware of any reason for such a difference. (The incompleteness of the toxicology database of 1080 overall is highlighted by the fact that no comparative toxicity values (such as LD_{50} values) appear to be available for these common strains of rat. Nevertheless, Chenoweth (1949) commented on the relative sensitivity of these strains observing that Sprague-Dawley rats routinely develop convulsions after exposure to 1080 while Wistar rats only do so rarely. The author attributed the difference in response do differences in acetate metabolism, but the Agency is unsure whether or not this reflects a modern biochemical understanding.)

B13.2.3 Developmental toxicity: Conclusion

The Agency considered the key study relating to developmental toxicity was the report by Eason et al 1999, based on MPI/Landcare (1988), and that this provided sufficient information on which to based classification of 1080 for developmental effects. No significance could be attributed to earlier negative, or mixed, results.

The Agency identified the following parameters:

Maternal

NOAEL 0.33 mg/kg bw/day

LOAEL 0.75 mg/kg bw/day

(based on effects on body weight, weight gain and food consumption)

Developmental (Foetal)

NOAEL 0.1mg/kg bw/day

LOAEL 0.33mg/kg bw/day

(based on forelimb abnormalities).

The Agency considers these data would support the classification of 1080 as 6.8B, but see overall conclusion below (section B13.4).

B13.3 Reproductive toxicity studies

B13.3.1 Data quality

When reviewing the reproductive toxicity studies, the Agency noted that no studies are available which complied with international guidelines for multi-generation toxicity tests in rats (such as OECD Test Guideline No 416).

The sub-chronic oral toxicity tests carried out in rats more recently by Wolfe (1988) and Eason and Turck (2002), were performed in the knowledge that the male reproductive system is one of the most sensitive target organ systems for 1080. Therefore, particular attention was given to the male reproductive system in those studies. Nevertheless, their study designs were only able to identify reduced spermatogenesis and target organ toxicity to the testes. They were not able measure reduced fertility.

The Agency identified only one study that demonstrated adverse effects on reproductive performance. The study in mink (Hornshaw et al 1986) demonstrated reduced fertility of males.

A multi-generation study would be highly desirable to confirm the reduced male fertility, and to investigate whether or not there are toxic effects on the reproductive process in female animals in addition to those found in the developmental studies.

Such a multi-generation study would also investigate whether or not 1080 is able to cause any effect on the female reproductive process. The oestrus cycling investigation in the 90-day study is totally inadequate in this respect.

Studies on the reversibility of the male reproductive toxicity are highly desirable.

Endocrine disrupting activity appears to have been excluded as a mechanism for 1080 toxicity to the reproductive systems, but the need for further studies as new techniques are developed should be considered. The mechanisms for the reproductive toxicity of 1080 are discussed in section B16.

The Agency did not consider that the lack of these studies were crucial or prevented decision making on this application. The reason for this conclusion is that where positive findings of some severity have been found (as in this instance), the results can be taken into account on the assumption that in a more thorough investigation clear reproductive toxicity would be demonstrated.

Nevertheless, the Agency is of the view that further investigations on the reproductive toxicity of 1080 are desirable due to severity of the effects found.

B13.3.2 Results

As noted above the studies reported here are not standard reproductive toxicity tests, but the Agency reported here those aspects of the target organ systemic toxicity relevant to the reproductive toxicity here. Other aspects of the studies are documented in detail under the target organ systemic toxicity (section B14).

Wolfe (1988)

1080 was administered by gavage to male and female Sprague-Dawley rats (20/sex/group) at doses of 0, 0.05, 0.20 or 0.50 mg/kg bw/day for 13 weeks.

Organ weight findings relevant to reproductive toxicity were significantly decreased absolute and relative testes weights in mid- and high-dose males. In these dose groups, changes in the testes included bilateral hypospermatogenesis with fusion bodies in the seminiferous tubules, and in the epididymides, immature and/or abnormal sperm and reduced sperm count.

Study conclusion

The key parameters resulting from this study were:

NOAEL for general toxic effects was 0.05 mg/kg bw/day.

LOAEL for reproductive was 0.2 mg/kg bw/day

(based on changes in the testes and epididymides in males)

Eason and Turck (2002)

Introduction

The Agency does not have the full study report of the investigation carried out for Landcare Research by MPI Research, Michigan, but carried out this assessment based on the summary in the paper, Eason and Turck (2002).

Results

Groups of 10 female and 10 male Sprague-Dawley rats received 1080 at 0.0, 0.025, 0.075 and 0.25 mg/kg bw/day by oral gavage for 90 days. Additional rats (10 of each sex) were dosed at the control and top dose levels and then observed for 56 days to monitor recovery.

Oestrous cycle activity was not found to be affected by treatment in female animals. Daily vaginal lavage of the female animals during the last three weeks of the study followed by microscopic examination of the cell types present was carried out. (The method is not one documented in any international guidelines of which the Agency is aware, however, it appears similar to that referred to in OECD Guideline 416. In that guideline, vaginal smears for determining oestrus cycling in rats is recommended early in the study to establish whether or not mating has occurred.)

Target organ effects relevant to reproductive toxicity were limited to the testes in the top dose group (0.25 mg/kg bw/day) males. All the top dose males were found to have small testes, graded as severe (1/10), moderate (8/10) and mild (1/10) by the authors. These findings were confirmed by the testes weights and the ratios of testes/body weights and testes/brain weights, and these reductions were statistically significantly. Males in lower dose levels were not affected. Mid-dose males (0.075 mg/kg bw/day) showed no effects at all, while the low dose males (0.025 mg/kg bw/day) had slightly raised mean testes weight in comparison to controls, presumably as a result of random statistical variation.

Sperm quality parameters were very severely affected in the top dose animals. There was severe hypospermia in the epididymides and severe degeneration of the seminiferous tubules. Severe decreases in the sperm counts were found. Sperm motility was reduced to 0%, and a large increase in percentage of abnormal sperm (to 99%) was reported.

In the recovery phase using only top dose animals and a control group, no improvement in the semen parameters was apparent in the treated animals in comparison to recovery controls. Similarly, the 10 treated recovery males showed no recovery of testes absolute or relative weights after 56 days.

Agency discussion

The Agency notes that OECD Guideline No 415 (one generation reproductive toxicity) (OECD, May 1983) states that the one complete spermatogenic cycle takes approximately 70 days in the rat. Therefore, the recovery period used in this study may be too short to establish whether or not recovery in male rats occurs after 1080 exposure. On the other hand, it is noted that the study gave no indication of any signs of recovery. Therefore, the Agency considered the evidence suggests that after prolonged exposure to 1080, recovery of the male testes and spermatogenesis is unlikely.

Study conclusion

NOAEL was 0.075 mg/kg bw/day.

LOAEL was 0.25 mg/kg bw/day

(based on effects in the testes and epididymides in male animals)

Hornshaw et al (1986)

Introduction

This paper reports on studies in mink and ferrets carried out in accordance with USEPA toxicity test requirement in place at the time. Reproductive toxicity tests were only carried out in mink.

Studies in mink

Results

Four male and 12 female animals were assigned to each group, a control, and three treatment groups fed diets containing 0, 0.05, 0 20 and 0.80 ppm of 1080 for approximately two months prior to mating. The animals were maintained on these diets for approximately a further four months.

A significant reduction in body weight was found in females receiving the top dietary concentration. The feed consummation measured during weeks 5–6 and 7–8 did not indicate a significant reduction in food intake even in the animals with reduced body weight gain.

Reproductive success was clearly reduced in the top dose animals in the study. Only two females out of 12 in the top dose group were mated successfully. (Success was judged by presence of live spermatozoa after mating.) Nine out of 12 of this group, although mated, were found to have no live spermatozoa. Of the two successfully mated females only one produced kits (mink offspring). The number of kits in this litter was consistent with the lowest dose group and control animals, however, these kits did not survive to the 3rd week post partum. (The paper does not indicate cause of death, which the Agency considers a serious deficiency in the report.) The fertility of mink fed a diet containing 0.8 ppm of 1080 was greatly reduced. Toxic effects to testes of the male animals, resulting in an absence of sperm, was the apparent cause of the reproductive failure,

since after mating, live sperm were not found in the majority of female animals at this dose level.

In the 0.20 ppm dose group, whelping was reduced to 7/12 (58%). This may be attributable to an effect of 1080. Although 10/12 of the females in this group had live spermatozoa, only seven produced litters. The number of kits per litter in the pregnant animal was reduced, four per litter, in comparison with six per litter in the control and 0.05 ppm groups. (In the 0.80 ppm, top dose group, one female had a litter of five kits, but these all died.) Although these differences may not be statistically significant, this suggests male fertility may be reduced at the lower dose rate (0.20 ppm).

At necropsy, the major finding was that the heart weights in the two top dose groups for the females were reduced. No data are provided for the male animals. In the 28-day dietary study in mink, reduced spleen, kidney and heart weight was reported in comparison to brain weights. Testes weights were reduced in what appears to be a dose related manner, but this was not reported by the authors to have reached statistical significance.

Agency discussion

The dietary NOAEL and LOAEL from this study were 0.2 ppm and 0.8 ppm respectively, but these cannot be clearly related to a dose rate for the male animals. The 1080 intake values for the mink were not determined separately for the male and female animals as they were caged together, and dietary intake was averaged across all animals in each dose group. The study reported the estimated 1080 intakes for the dose groups at Weeks 7–8 as 0.05 ppm (0.01 mg /day), 0.20 ppm (0.04 mg/day) and 0.80 (0.15 mg/day). No values on a per kilogram body weight basis were provided. Based on the bodyweight of the male animals (in which the toxic effect were found) at the end of the study, this indicates the dose rate on a per kg basis would have been approximately 0.006, 0.024 and 0.08 mg/kg bw/day respectively. These figures are very approximate. The male animals would consume a higher proportion of the diet than the females, and the weight of the animals at the end of the study would be higher. Therefore, the dietary intake on a per kilogram basis earlier in the study is likely to be higher than this estimate.

This study is unique in providing some evidence of reproductive effects that may be due to effect on the maternal animals. All the pups in the litter from the single female mink which was successfully mated at the top dose, died after parturition (of unstated causes).

Studies in ferrets

Although no reproductive performance tests were carried out with ferrets, in the 28-day dietary feeding study, young male ferrets were found to have lower testes weights when treated with 1080 than control animals, even though the organs were at an early developmental stage.

Study conclusion

Only tentative NOAEL and LOAEL values were available for the male mink in this study, but the estimates are:

NOAEL at 0.2 ppm in diet, equivalent to approximately 0.024 mg/kg bw/day

LOAEL at 0.8 ppm in diet, equivalent to approximately 0.08 mg/kg bw/day

(based on reduced fertility and absence of spermatozoa after mating)

Other studies on reproductive toxicity

Introduction

Early studies give substantial support for the target organ effect of 1080 of the testes, showed histological effects on the organ and reductions in spermatogenesis. However, effects on reproductive performance were not demonstrated. Although the data are not suitable as regulatory purposes, the confirmation of effects found in the studies above was considered by the Agency to be of significance.

Smith et al 1977

Smith et al, 1977 attempted to verify a report (the authors cited Mazzanti et al, 1965) of effects on the testes after an 11-day exposure to 1080. They carried out a chronic exposure investigation in male Sprague-Dawley rats using 1080 in drinking water for up to 126 days. The drinking water concentration was 5 ppm fluoroacetate (26 ppm as sodium fluoroacetate). After this exposure the histological examination of the testes showed advanced atrophy with loss of the normal ordered structure, with gaps and irregular shapes in place of normal seminiferous tubules. Both functional cells and spermatozoa were absent.

The water intake for the rats is not reported. An estimate of the dose rate based on the standard drinking water intakes for rats (Fairchild et al 1977) was done, but the result (3.25 mg/kg bw/day) seems too high to be correct for a study of this duration. (Drinking water intakes vary greatly with age in relation to body weight, so the estimate is very uncertain.)

Sullivan et al (1979)

Sullivan et al (1979) studied Sprague Dawley rats over relatively short period in an attempt to investigate reversibility of the testicular effects. The rats received 2.2, 6.6 or 20 ppm of 1080 in drinking water for seven days. The study reported the average 1080 intakes at these dose levels were 0.07 mg/kg bw/day, 0.18 mg/kg bw/day and 0.71 mg/kg bw/day, respectively. Testes weight was reduced in the 6.6 and 20 ppm groups and was associated with morphological damage. Cellular changes in testes common to all treatment groups consisted of altered appearance, reduced numbers of spermatids, and presence of both spermatid and spermatocyte giant cells. The two higher dose groups showed marked seminiferous tubule atrophy. Some animals were examined up to 21 days after the end

of treatment in an attempt to investigate reversibility of the testicular effects. The authors reported regeneration of the seminiferous tubules in the 2.2 ppm exposure group after seven days (after end of the exposure period), but even at 21 days the recovery was not complete in the two higher dose groups.

Amer et al 1986

A subsequent study had as its focus the question of whether or not the effects on spermatogenesis are reversible. The study used a surgical technique (termed by the authors "vasocystostomy") in rats to assess reversibility of the testicular effects of 1080 (Amer et al 1986). The output from the testes in the Charles River rats was redirected to the bladder so that sperm counts in the collected urine could be studied in real time in the live animals during, or after, exposure to test substances. 1080 was used as a known testicular toxin in an attempt to validate the method. The method is not recognised in test guidelines, and has not been verified subsequently as far as the Agency can determine.

The rats were exposed to 20 ppm of 1080 in drinking water for seven days, equivalent to approximately 2.5 mg/kg bw/day (as an approximate estimate). In all treated rats, an increase in sperm count occurred shortly after treatment, but after treatment was completed, the sperm counts rapidly declined to practically zero. No explanation for the initial increase was offered by the authors. (A possible explanation is that the increase in sperm count during treatment may be the result of death of cells in various stages of differentiation and being released into the lumen).

The authors suggested some recovery was evident in two of the five rats according to the sperm counts. Histological investigations at the end of the study indicated there were patchy degenerative changes in the testes, including multinucleated spermatogonia. However, the testes from animals in which recovery was suggested by the sperm counts, showed histological findings that were not qualitatively different.

The Agency considers that the lack of demonstrated reliability of the method makes clear interpretation of the data impossible and concludes that the evidence for reversibility of the testicular toxicity in this report is inconclusive.

Wickstrom et al (1997)

Introduction

Wickstrom et al (1997) carried out an investigation into the general health and reproductive performance in sheep following a **single**, high, sub-lethal dose of 1080. The Agency notes the study design was drawn up to indicate whether or not an accidental acute exposure to 1080 in sheep would be likely to be detrimental to the animals reproduction if they were close to term.

Results

The report of the first stage (Meikle et al 1996) was not available for review by the Agency. The report of the second stage indicates that at the start of the study, 52 ewes were exposed to 0.25–0.3 mg/kg bw 1080 in a single dose. Almost half (21) of the sheep died, which demonstrated this was a high sub-lethal dose (for the surviving animals which formed the study population).

Ten of the surviving animals were killed at 14 days and examined. No 1080 residues or haematological or biochemical 1080-related abnormalities were detected. The health and reproductive performance of 21 remaining survivors was then followed for two years (without further 1080 exposure). These animals were maintained under normal farming conditions. This included the two lambing cycles up to the second weaning.

The study claims the ewes demonstrated no adverse effects on:

- body weight gain
- wool production
- lambing performance through to weaning, including lambing percentage, lamb birth weight, lamb survival and growth rate.

At necropsy after two years, the treated ewes showed occasional small foci of interstitial fibrosis in the cardiac (heart) muscle, primarily in the left ventricular free wall. (The findings are discussed further in section B14.)

Agency conclusion

The study does not provide information that is useful for assessment of reproductive hazards, because the animals were administered a single dose and for the surviving animals all the poison would have been excreted at the time of the reproductive processes.

O'Connor et al (1999)

Results

O'Connor et al (1999) studied the effects of a single or small number (three) of doses of 1080 in pregnant ewes. Groups of 20 Perendale ewes, non-pregnant or pregnant with twins, were given either a single dose of 0.25 mg/kg bw of 1080 or 0.05 mg/kg bw (daily) for three days. These were high acute and sub-acute doses. The original intention had been for the repeat dose animals to receive 0.05 mg/kg bw (daily) for five days, but due to significant deaths on the third day of treatment, no further doses were administered.

Agency discussion

The authors claim there was an increase in toxicity of 1080 to pregnant ewes in comparison to non-pregnant ewes, due to higher bioavailability and higher serum concentrations in the pregnant ewes. This suggestion would support the finding of Annison et al (1960) that sheep were more

sensitive than expected to repeated sub-lethal doses of 1080. The toxicokinetics, serum analyses and histological findings are discussed in section B15. Since the pregnant ewes showed no significant effects on lamb percentage, lamb survival or lamb weights (until market), the Agency concluded the higher toxicity of 1080 in pregnant ewes is uncertain.

The Agency notes that in order for these findings to inform the developmental toxicity of 1080 in sheep, it would be necessary for the treatment to have occurred during the period of organogenesis. In fact a particular aim of the study was to determine whether or not pregnant ewes **close to term** are more sensitive to 1080 than other ewes. Therefore, treatment of the ewes occurred only two weeks prior to lambing, and the study is not suitable for assessing developmental effects. The lack of effect on lambing percentages indicates that at these doses, there was no selective foetotoxicity in sheep from sub-lethal acute doses late in gestation.

Agency conclusion

The Agency concludes the study provides some evidence in support the view that pregnant sheep are more sensitive to 1080, but that the finding is inconclusive. While the study did not demonstrate foetotoxicity late in gestation from a high sub-lethal dose, the Agency did not consider the design of the study was suitable for determining whether or not 1080 is a developmental toxicant or is foetotoxic in sheep.

B13.3.3 Conclusions on reproductive toxicity effects

The sub-chronic toxicity studies in the rats identified reduced relative and absolute testes weights, associated hypospermatogenesis, reduced sperm quality and histological findings in testes.

In addition, the study in mink demonstrated reduced fertility in males due to exposure to 1080. Although mink is an unusual animal model for human toxicity testing, this information provides crucial supporting evidence, due to the incompleteness of the dataset. Reduced testicular weight was also shown in ferrets.

In 90-day studies on rats, the effects on the male reproductive system were associated with the following parameters:

NOAEL was 0.075 mg/kg bw/day

LOAEL was 0.25 mg/kg bw/day

(based on effects on the testes and epididymides in male animals)

Effects on semen quality and the testis have been demonstrated in rats after a single and small number of acute doses. In longer term tests, the effects are very clear and reversibility of the effects has not been demonstrated. The Agency concludes the data suggest the effects are irreversible.

Although the data are incomplete, given the magnitude and consistency of the effects reported in three separate lower mammalian species, the Agency considers severe reproductive effects on the testes have been demonstrated. Although no evidence is available relating to effects on exposed humans, the effects are severe and are considered likely to be irreversible, so a 6.8A classification was applied.

The Agency considers these data support the classification of 1080 as 6.8A.

B13.4 Agency overview: Reproductive and developmental toxicity (6.8)

The Agency considers the overall database consistent in demonstrating both developmental and male reproductive toxicity effects. The relevant NOAELs (no observed adverse effect levels) and LOAELs (lowest observed adverse effect levels) are listed in Table B6, together with the relevant classification derived from consideration of the *User Guide to HSNO Thresholds and Classifications*.

Table B6: NOAEL and LOAEL values for reproductive and developmental toxicity and proposed classifications

Study type	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Classification proposed
Fetal effects (developmental) (rats)	0.1	0.33	6.8B
Male reproductive toxicity (rats)	0.075	0.25	6.8A
Male reproductive toxicity (mink)	0.024 (tentative)	0.08 (tentative)	(supporting information only)

The Agency notes that the more severe of these classifications drives the overall classification for developmental and reproductive toxicity.

- ***Classification of 1080: Developmental and reproductive toxicity: 6.8A***

B14 Target organ systemic toxicity (6.9)

B14.1 Introduction

Target organ systemic toxicity can be triggered both from acute and sub-chronic/chronic data. In the case of 1080, the Agency considered the need for a target organ toxicity classification on the basis of toxic effects from both acute and sub-chronic exposures. (Note: In the case of target organ systemic toxicity expert judgment and human data can be used as a basis for classification (without the limitations referred to under 6.1 inhalation above).)

B14.2 Target organ classification for acute toxicity

B14.2.1 Discussion

Animal data

Animal data relating to acute toxicity are discussed in section B3.

The toxic effects of 1080 causing lethality are classified under 6.1, but single doses of 1080, not sufficient to be lethal, are known to cause both short-term and long-term impairment of function of an organ as a result of the exposure. Since such effects are not classified directly under 6.1, a target organ classification may be appropriate.

Target organ toxicity in acute toxicity tests is clearly demonstrated by the effects reported, such as seizures, heart beat disorders (arrhythmias), heart failure, and biochemical abnormalities such as raised serum citrate and glucose levels, metabolic acidosis, and reduced serum calcium (Chenoweth 1949; Atzert 1971). These toxic effects may occur in animals that subsequently recover, so they are not captured by the 6.1 classification and are appropriate for 6.9 target organ classification.

More recently, more subtle effects were demonstrated in sheep following a single or small number of acute or sub-acute doses respectively. Wickstrom et al (1997) found that after exposure to a high, sub-lethal dose of 1080 (a single dose of 0.25 mg/kg bw or 3 doses of 0.05 mg/kg bw/day), sheep were found to have histological heart abnormalities and this was the only abnormal finding in the interim sacrifice animals at 14 days. Animals examined at necropsy two years later (after only this single 1080 exposure), during which time they successfully raised two lambs, were found to have occasional small foci of interstitial fibrosis, most prominently in the left ventricular free wall. The authors noted these findings appeared to be without clinical significance.

The Agency considers the toxicological significance of histological effects in the heart are debatable, although it seems likely that they are the result of 1080 exposure. Taken in isolation they would not justify classification.

Human findings

Reports relating to acute human toxicity are discussed in section B17. There is clear evidence from human clinical cases that high acute doses of 1080 can produce temporary organ damage to the heart and brain after a single exposure. Such findings are reported in clinical toxicology reports, usually of accidental or suicidal poisonings. One clinical report (Trabes et al 1983) identified an acute exposure to 1080 that resulted in long-term, probably permanent, brain damage. In most of the reports, the impairment related to apparently reversible effects (Chi et al 1996; Gajdusek and Luther 1950), although frequently the papers do not indicate whether long-term follow-up took place.

One element of uncertainty such studies raise is whether or not the target organ effects in humans could occur without lethality in the absence of intensive treatment of the patient. It is likely that many patients who suffer severe long-term effects would have died if medical treatment had not been received. The Agency does not consider that this issue should preclude the classification on the basis of target organ effects from acute human exposures.

B14.2.2 Conclusion for acute target organ toxicity

The Agency considered that acute exposures to 1080 in humans and animals may give rise to both reversible and irreversible adverse, target organ effects. These effects are severe and were found after human acute exposures, so the 6.9A classification is appropriate for 1080.

B14.3 Target organ classification for sub-chronic toxicity

B14.3.1 Introduction

The ideal study for determining a 6.9 classification is an oral 90-day toxicity study in rodents. Due to the known reproductive effects particularly on male animals, some of these studies report information related to reproductive effects. Coverage of the effects on reproductive organs relevant to the 6.8 classification is discussed in section B13, but general target organ effects are discussed here.

B14.3.2 Data quality of sub-chronic toxicity studies

Two sub-chronic toxicity studies in rats have been carried out relatively recently in accordance with international guidelines. Indeed these studies in some respects exceeded the guideline requirements, because the study design was modified to address particular toxicity concerns from 1080.

The Agency notes that sub-chronic studies have only been carried out in rats.

The Agency does not consider the lack of studies in other species represents a significant data gap for 1080 that prevents a decision on this application to be made.

B14.3.3 Results

Eason and Turck (2002)

The Agency does not have the full study report of the study carried out for Landcare Research by MPI Research, Michigan, and used the summary information from the above published paper.

Groups of Sprague-Dawley rats (10 male and 10 females per group) received 1080 at 0.025, 0.075 and 0.25 mg/kg bw by oral gavage for 90 days. An additional group of rats, 10 of each sex, was dosed at the top

dose level for the duration of the study and then returned to the control diet. These animals and a control group were then examined after a 56-day recovery period.

There were no general clinical adverse effects (including behavioural observations), nor any significant changes in body weight, overall, although there were periodic differences in males and females at the top dose. These occurred without any consistent pattern, so were not considered treatment related.

Clinical chemistry and erythrocyte parameters also had only incidental differences at particular time periods not considered treatment-related by the study authors.

Investigation of the effects on the reproductive system (and specifically the toxic effects on the testes) found in this study are discussed in section B13.

The other major target organ was the heart. Cardiomyopathy was found in both sexes at the top dose level only. The female rats showed signs of recovery from heart histological effects during the 56-day recovery period. This recovery was seen in only some of the male animals.

NOAEL was 0.075 mg/kg bw/day.

LOAEL is 0.25 mg/kg bw/day

(related to effects on the heart, cardiomyopathy)

Wolfe (1988)

1080 was administered by gavage to male and female Sprague-Dawley rats, 20 animals of both sexes per group at the following dose rates: 0 (control), 0.05, 0.20 or 0.50 mg/kg/day for 13 weeks.

Individual body weight measurements, food consumption and opthalmoscopic examination indicated no signs of toxicity. During weeks 4 and 13, blood samples were collected and examined for haematology (hemoglobin, haematocrit, leukocyte, erythrocyte, platelet, reticulocyte and differential leukocyte counts; and cell morphology). Complete serum chemistry analysis was done including serum fluorocitrate measurements. Following sacrifice at week 13, all animals were necropsied, after which organ weights were recorded, tissue samples taken, and complete histopathological examinations performed.

Dose-related effects in organ weights included increased absolute and relative heart weights in mid- and high-dose females and high-dose males. Also significantly decreased absolute and relative testes weight in mid- and high-dose males (see section B13). Absolute spleen weights were significantly reduced in top dose males. Relative spleen weights in top dose males and relative and absolute spleen weights in top dose females were reduced, but not to a statistically significant degree.

Histological effects included changes in heart tissue consisting of sub-acute, minimal inflammation, but were not found to be dose-related in this study. (See section B13 for histological effects on testes.)

One female rat (in the high-dose group) that died before week 13 could not be classified as an accidental death. Four high-dose female rats exhibited convulsions on study day 79 (week 12), with no recurrences for the remainder of the study.

Clinical chemistry was examined at week 4 and week 13. Fluorocitrate levels were significantly increased after four weeks in the high-dose males and after 13 weeks in both the mid- and high-dose groups of both sexes. The Agency notes that there is no reference to the isomer of fluorocitrate analysed in these tests, so assumed the chemical method used did not distinguish between isomers. As would be expected, the treated animals had much higher levels of fluorocitrate in serum. An unexpected finding was that the serum of control rats contained low, but detectable, concentrations of fluorocitrate, which appeared to be above the margin of error from zero. The study report made no comment on this finding, although the Agency considered it unexpected. Ideally, analyses of serum in control animals from other studies would have been carried out to confirm the finding, in particular, to exclude the possibility of cross contamination. Serum glucose was not raised except in one dose group at week 4. Citrate levels in serum were not reported. Other findings were considered incidental.

The treatment-related effects triggering 6.9 target organ toxicity in this study were increased heart weight (without associated histological changes) in mid and top dose females and top dose males. Absolute spleen weights were reduced in top dose males. The Agency notes similar findings were reported in the 28 days study in mink (see section B13.)

NOAEL 0.05mg/kg bw/day

LOAEL 0.2 mg/kg bw/day.

(based on increased absolute and relative heart weights)

B14.4 Agency conclusions for target organ systemic toxicity

The Agency considered 1080 should be classified as a 6.9A target organ systemic toxicant, on the basis of its acute metabolic effects, effects on the heart, toxic findings such as effects on cellular energy production, and seizures. In relation to the sub-chronic target organ effects (on organs other than the male reproductive system), the primary target organ is the heart. While some studies show histological changes, others report increased heart weight only, but these effects appear after both acute and sub-chronic exposures.

The resulting end points were compared with the criteria in the *User Guide to Thresholds and Classifications Part VI*, p 91, (iii) Single exposure target organ effects. The acute, single doses that can produce the acute target

organ effects referred to above are below the threshold given (≤5 mg/kg) for 6.9A, so this classification is appropriate.

The resulting end points were compared with the criteria in the *User Guide to Thresholds and Classifications* of Part VI, p 92, (iv) Repeated dose target organ effects. The lowest LOAEL in is 0.2mg/kg bw/day for the cardiac effects in the Wolfe (1988) study. This value is well below the threshold for assigning 1080 the 6.9A classification, which is 10 mg/kg bw/day in a 90-day study, so this classification is appropriate.

Aside from the dose rat ranges from the *User Guide to Thresholds and Classifications,* the Agency noted that the human toxic effects discussed briefly above (and in section B17) would trigger 6.9A, target organ systemic toxicity for 1080.

The NOAEL (no observed adverse effect level) and LOAEL (lowest observed adverse effect level) values for target organ systemic toxicity are listed in Table B7.

Table B7: NOAEL and LOAEL values for target organ systemic toxicity

Study type	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)
Ninety-day study rats (Eason and Turck 2002)	0.075	0.25 (cardiomyopathy)
Ninety-day study rats (Wolfe 1988)	0.05	0.2 (heart weight)

The Agency, therefore, concludes classification for 1080 applying both for acute and chronic exposure is 6.9A.

- ***Classification for 1080: Target organ systemic toxicant: 6.9A***

B15 Toxicokinetics and metabolism

B15.1 Toxicokinetics

1080 is readily absorbed by mammals following oral administration. It is noteworthy that 1080 has similar toxicity parameters for oral and injection. This is unusual and indicates rapid and complete absorption from the gastrointestinal tract (Chenoweth 1949).

Absorption through mucous membranes, the respiratory tract and broken skin is reported as rapid, but slower through intact skin (Atzert 1971). These conclusions have been reached on the basis of the acute toxicity, rather than absorption data. No dermal absorption studies in either humans or animal models were identified by the Agency. One source reported that absorption through the skin may be higher if it is damp or sweaty (Rammell and Fleming 1978, cited Department of Health, 1967). The Agency considered this may be precautionary advice from health officials at the time, rather than a statement relating to known skin absorption of the compound.

No inhalation toxicity or inhalation absorption studies have been located, but a single incident of human exposure to dust containing 1080 suggests rapid absorption from the respiratory tract in humans (Williams 1948).

1080 has high water solubility so rapid distribution, at least in extra-cellular compartments within the body, would be expected. Since 1080 is ionised in solution, this may be expected to hinder movement across cellular membranes, into cells and organelles (such as mitochondria). However, distribution studies indicate 1080 passes relatively freely through membranes and into cells (Sykes et al 1987). Atzert (1971) commented that the molecular size difference between the fluoroacetate ion and acetate ion is small. The Agency concluded that distribution of 1080 across membranes may be facilitated by transport mechanisms for acetate.

In rats, the average percent distribution after a range of acutely toxic doses (below and above the LD_{50} value) was 7.5% for plasma (7.1% was reported in the paper in error), 5.1% for liver and 1.7% for kidney in rats (Egekeze and Oehme 1979b). The time after dosing at which these percentages were determined varied. Analysis of the tissues for each rat was timed depending on the latency period determined from each animal's clinical signs. The Agency considered this an unusual analytical approach, as the time at which the data was measured varied between individual animals.

By use of fluoroacetate carrying a radioisotopic label on the fluorine (^{18}F fluoroacetate), Sykes et al (1987) were able to demonstrate relative uniform clearance from soft tissues with consistent half-lives in the range 1.7–2 hours. The finding supports the view that fluoroacetate is readily distributed around the body, between vascular compartments and within tissues.

Excretion of unchanged 1080 in urine was reported to be important in a range of animals (Chenoweth and Gilman 1946). The toxicokinetics of 1080 have been investigated in rats by dosing of Sprague Dawley rats, by injection, with radioisotopic labelled 1080 (2-^{14}C sodium monofluoroacetate) (Gal et al 1961). Administration of labelled material enables the distribution and excretion of the substance in the rat to be determined. This study indicated that approximately one third of an absorbed dose was usually excreted unchanged in urine in Sprague Dawley rats. A lower proportion of an absorbed dose appeared to be excreted in urine after lethal doses (Gal et al 1961).

The remaining (approximately two thirds) of the 1080 is metabolised. Fluorocitrate is produced and this is also excreted in urine. Studies using carbon radioisotope labelled 1080 (2-^{14}C sodium monofluoroacetate) indicated a relatively small proportion (1.4%) of the carbon from this labelled compound is excreted as carbon dioxide (CO_2). The researchers found that there was considerable individual variation on this proportion between rats of the same age, strain and sex under identical dietary regimes. The variation in the proportion excreted as CO_2 was between

about 0.26% and 2.5%. Data on the proportion fully metabolised in other laboratory species were not found.

In carbon-labelling studies, after four days, only a very small proportion of the carbon label (0.2%) remained to be excreted. This demonstrates that very little 1080 remained in the animal. Indeed, it is likely that this labelled carbon which had not been excreted, had been incorporated into metabolic products in the body and was no longer present as 1080 (or more accurately fluoroacetate).

In sub-chronic (90-day) studies in Sprague Dawley rats (Eason and Turck 2002), 1080 concentrations in serum and urine were measured. The mean urinary 1080 levels, a day before sacrifice, after doses rates of 0.025, 0 075 and 0.25mg/kg bw/day were 0.006, 0.032 and 0.059 mg/ml respectively. While dose-related, these were noted not to be proportionate to dose levels received. These concentrations were not adjusted to take into account the creatinine concentration and urine volume for each animal, as these parameters were not measured.

In the same study the plasma concentrations 1 and 12 hours after dosing on days 10 and 77 appeared to increase in a dose-related manner. There was no significant difference between the concentrations measured on days 10 and 77. The plasma concentrations at 1 and 12 hours after dosing were approximately, 0.036 and 0.005 mg/ml (low dose), 0.086 and 0.022 mg/ml (mid dose) and 0.25 and 0.075 mg/ml (top) dose respectively. This indicates that at these dose rates, rats do not accumulate 1080, despite repeated dosing for 90 days.

Little data are available on toxicokinetics of 1080 in other species. Eason 1994 studied the toxicokinetics of 1080 in nine sheep and two goats after treatment of the animals with 0.1mg/kg bw of 1080. (The LD_{50} values for the sheep were quoted as 0.4 and 0.6 mg/kg bw in sheep and goats respectively.) The maximum serum concentrations were achieved at an average of 2.5 hours (range 0.5–4 hours) in sheep and at 0.5 or 1 hour, respectively, for the goats. The plasma half-life of 1080 in sheep was on average 10.8 hours (range 6.6–13.3 hours) and 5.4 hours in goats (3.9 or 6.9 hours for the two animals). By 96 hours, only trace amounts of the parent compound (fluoroacetate) could be detected in any tissue in sheep.

Toxicokinetic investigations were also performed in pregnant and non-pregnant ewes (O'Connor et al 1999). Animals given a single dose of 0.25 mg/kg bw of 1080 (a high acute dose) reached their peak serum concentrations of the parent compound one day after treatment. By day 3 the concentration had fallen to levels not significantly different from day 1 (prior to dosing). Comparison of pregnant and non-pregnant ewes showed greater bioavailability of 1080 in pregnant animals. (Higher maximum serum concentrations were demonstrated in pregnant animals.) In groups given multiple doses of 0.05 mg/kg bw of 1080 daily for 3 days, the peak serum concentration was achieved on day 2. Thus, there was little cumulative effect of repeated doses.

The half-lives in a small range of other species were reported by Eason et al (1994). The abstract provided with the application, quotes elimination half-lives for 1080 at near lethal doses of 1080 were as follows 1 hour (rabbit), 2 hours (mouse), 11 hours (sheep) and 5 hours (goat). The Agency notes that the values for goats and sheep are consistent with those given above.

The elimination half life in the small rodents appear to be much lower than for the larger domestic animals, which is a common finding for many substances, due to the higher metabolic rate of small rodents in comparison with higher mammals. Nevertheless, at near acutely toxic levels in sheep, no accumulation of 1080 or its metabolites was reported.

The Agency was not able to locate information for the half-life or distribution of 1080 and its metabolites in humans. Although investigations of human poisoning cases have been undertaken (section B17), very few of the clinical reports involved analysis of human tissue for 1080 and its metabolites.

B15.2 Metabolism

B15.2.1 Krebs cycle intermediates

1080 is metabolised at the cellular level in the bodies of all aerobic mammals to fluorocitrate through a number of metabolic steps. Firstly, fluoroacetate is converted into fluoroacetyl-CoA (fluroacetyl coenzyme A) by acetyl-CoA synthetase. Fluoroacetyl-CoA then reacts with oxaloacetate under the influence of the Kreb's Cycle enzyme, acetyl-CoA transferase, to generate fluorocitrate.

Metabolism of 1080 is of particular significance because it is the formation of fluorocitrate, which is necessary to produce the toxic effects of 1080 poisoning. Chemically there are four possible fluorocitrate isomers. In biological systems the conversion by an enzymatic process, produces only one stereoisomer, L-erythrofluorocitric acid. It is this particular isomer which is the active (toxic) metabolite. Savarie (1984) defines the active isomer as the 2R, 3R (-) erythrofluoroacitric acid. One important implication of this is that toxicological/metabolic studies using fluorocitrate from chemical synthesis do not accurately reflect the situation in the animal following exposure to 1080 itself. The chemical synthesis produces a range of the stereoisomers (since the configuration of the active site in the biological enzyme is not replicated), so the when chemically derived fluorocitrate treatments are used, these are not equivalent to the fluorocitrate produced in the animal. The exposure in the organisms is actually to a smaller dose of the active isomer and this is acknowledged in at least study (Tremblay et al 2005).

Further metabolism of the active isomer via the Kreb's cycle is impossible due to its binding to the aconitase enzyme, without being a successful substrate. Fluorocitrate levels rise in the serum of the animal and it is

excreted in urine (Bosakowski and Levin 1986). Nevertheless, metabolism by pathways other than the Kreb's Cycle cannot be excluded.

Only a relatively small proportion of an administered dose of 1080 is converted to fluorocitrate. This has been commented on commonly, particularly in early papers, as it is a relatively unexpected finding. Nevertheless, a sub-chronic rat study has clearly document raised fluorocitrate concentrations in serum and urine (Wolfe 1988).

B15.2.2 Other metabolic pathways

In the study of the toxicokinetics of 1080 using radioisotopic labeled 1080 (2-^{14}C sodium monofluoroacetate) (Gal et al 1961) discussed above, the metabolism of 1080 in the rat was studied with particular focus on the fate of the carbon-fluorine bond (hence the label was on the second carbon atom). It was expected that the carbon-fluorine bond would be resistant to breakdown within a biochemical system. In fact, the study demonstrated that biological systems have the ability to break the carbon-fluorine bond. As referred to under Toxicokinetics (section B15.1) this was demonstrated by the production of labelled carbon dioxide, although in relatively small quantities.

The release of fluoride from 1080 has been verified more definitively using ^{18}F fluoroacetate, 1080 with the radioisotopic label on the fluorine atom (Sykes et al 1987). This study in mice showed unambiguously that some fluorine from 1080 is released as fluoride. About 2 hours after administration, approximately 26% of the labelled fluorine in serum was present as fluoride (not ^{18}F-fluoroacetate). This confirms the result reported above demonstrating de-fluorination by the release of carbon labelled CO_2 (Gal et al 1961). It also confirms the availability of 1080 derived-fluorine in the fluoride balance studies using rats fed low-fluoride diets (Miller and Phillips 1955; Smith 1977) or fluoroacetate in drinking water (Smith et al 1977). As would be expected a proportion of the fluoride release is retained in the bone.

However, some of the findings of Sykes et al (1987) appeared inconsistent with other work. In particular, they claimed a high proportion of labelled fluorine is excreted as fluoride rather than ^{18}F-fluoroacetate, and this appears to have resulted in a misleading statement about the proportion of 1080 excreted unchanged (Rammel and Fleming 1978). Nevertheless, Sykes et al 1987 provided pivotal information of release of fluoride from fluoroacetate. They reported that the de-fluorination of fluoroacetate is associated with glutathione –S-transferase (or a related enzyme) in the liver or kidney (citing 14) and (15) from Soiefer and Kostyniak 1983). The authors (Soiefer and Kostyniak 1983) investigated the metabolism of 1080 in Swiss Webster Mice (CFW) mice. They ranked the mice organs in their ability to de-fluorinate 1080 as follows: liver> kidney >> lung > heart > testes. The ability of both the liver and kidney to carry out this critical (detoxification) step was substantially greater than the other organs. It is noteworthy that the heart and testes were least able to do so. They

reported no activity of this enzyme system in the brain. The kidney was active at a rate approximately one fifth of that of the liver. Much lower activities were reported for the other tissues ranked above. Reduced glutathione enhanced the activity, but other reduced sulphur compounds 2-mercaptoethanol, dithiothreitol or cysteine did not enhance de-fluorination. Activity was enhanced at higher pH in the range 6–9. Subsequent sources have confirmed fluoroacetate is conjugated by glutathione (Clarke 1991; Tecle et al 1989).

The role of glutathione in metabolism and defluorination of fluoroacetate in possum (*Trichosurus vulpecula*), both Western Australian and South Australian animals, was investigated primarily in an attempt to explain the relative resistance of the West Australian animals (Mead et al 1979). The South Australian animals were able to de-fluorinate fluoroacetate in a similar manner to the West Australian animals. This work indicated that it is fluoroacetate itself, not fluoroacetyl-CoA, which is de-fluorinated.

Although the liver is a highly metabolically active organ, it has been noted that the metabolism of acetate and acetyl CoA is often not done in the liver, but in other parts of the body. This indicates that fluoroacetate and fluoracetyl-CoA generated in the liver may pass to other parts of the body before further metabolism. This is assuming that the size difference for the fluorinated form does not affect the transport mechanism as discussed above.

One metabolic product was identified as being an amino acid, although full characterisation was not possible (Gal et al 1961; Schaefer and Machleidt, Biochim Biophys Act 1971). Given the similarity of the atomic size of fluoride and hydrogen, it is possible that a fluorinated amino acid may be incorporated into proteins. Whether this is responsible for any toxic effects is unknown, but it may explain the difference in responses seen in different species. The Agency found no more recent work confirming the finding, and considers it needs verification using modern analytical techniques. Clarke (1991) also refers to the interaction of 1080 metabolites on amino acid metabolism.

Gal et al (1961) also showed using labelled carbon that 0.1–0.5% of the label was detectable in the lipid fraction consisting of fatty acids and cholesterol in the liver. This did not increase with time, suggesting the conversion only occurred before the energy sources in the cell became depleted. The cholesterol elsewhere in the animal did not contain a significant incorporation, suggesting metabolic differences in the liver (Gal et al 1961).

De-fluorination of (-) erythrofluorocitrate, the toxic isomer produced in cells from fluoroacetyl-CoA, by aconitase was been reported (Clarke 1991) releasing the fluorine as fluoride. This finding appears to contradict the generally accepted mechanism of toxicity of fluorocitrate. De-fluorination would appear to be a detoxification mechanism, but it may damage the enzyme in the process.

As discussed under target organ toxicity (Wolfe 1988) reported that a background level of fluorocitrate was detectable in the control group rat (which would have received no 1080 in their diet). The Agency considered this an unexpected finding which needs confirmation, before it is consider proven. It may indicate that a small amount of fluorocitrate is produced from dietary fluoride, in the rat, but such a conclusion is not justified on the basis of a single, unconfirmed finding.

B16 Mechanism of action and related issues

B16.1 Acute toxicity

B16.1.1 Basic mechanism

1080 is a very toxic compound, because in mammalian systems (and many other animals and plants) it is readily metabolised to a toxic isomer of fluorocitrate (strictly speaking a particular isomer (-) erythrofluorocitrate, see above), which interferes with a key biochemical pathway, the Kreb's Cycle. The original understanding of the mechanism was that the fluorocitrate isomer inhibited an enzyme, aconitase, from the Kreb's Cycle. Aconitase isomerases citrate, so this conclusion was consistent with the finding that citrate levels are raised after 1080 poisoning. (An isomerase is an enzyme which changes the structural isomer of a molecule. The change is necessary for the next step of the Kreb's Cycle to occur.) The effect of this blockage would be to prevent the operation of the Kreb's Cycle and the release of energy (primarily as ATP) in cellular aerobic respiration. It was assumed that aconitase was reversibly bound to the fluorocitrate molecule, preventing the metabolism of citrate.

Subsequent studies have demonstrated that other effects may also be important. Inhibition of a citrate carrier protein in the mitochondrion has been demonstrated (Kun et al 1977). Kun et al were able to show that one particular (-) erythrofluoroacetate-glutathione complex inhibited the formation of citryl glutathione thio ester. (Glutathione is a tripeptide found most commonly in the liver and kidney and is important for metabolism of some toxic chemicals. It contains a lot of the sulphur-containing aminoacid, cysteine.) This more complex explanation of mechanisms for fluoroacetate and fluorocitrate toxicity has been endorsed by more recent reviews (Savarie 1984; Clarke 1991).

The suggestion that aconitase can de-fluorinate (-) erythrofluorocitrate, the toxic isomer produced in cells from fluoroacetyl-CoA, is a more fundamental proposal and appears to contradict the original proposed mechanism of toxicity (Clarke 1991). This would suggest that rather than the blocking of aconitase, fluorocitrate causes the metabolic disruption of the Krebs Cycle by disrupting citrate transport as proposed by Kun et al (1977). It is important in this context to note that the toxic fluorocitrate isomer, while a substrate for the aconitase, would give rise to the usual reaction products, and most critically the aconitase enzyme's active site may be inactivated in the process.

Rather than reaching a firm conclusion, the recent review (Clarke 1991), noted the on-going mechanistic uncertainties and appears to confirm that relatively little work has been done on the mechanism of fluoroacetate or fluorocitrate toxicity since the 1970s. The overall conclusion was that the small quantity of fluorocitrate produced from fluoroacetate is consistent with an enzyme or transport inhibition. It appears that the active isomer prevents the transportation of citrate in the cell thus preventing the aerobic respiration and replenishment of energy stores.

The Agency considers that while there may be uncertainty relating to the precise biochemical mechanism for acute poisoning by 1080, the overall conclusion that fluorocitrate disrupts the Kreb's Cycle, severely reducing the ability of cells to utilise oxygen in order to replenish energy at the cellular level, remains a valid understanding of the mechanism overall.

B16.1.2 Mechanism of toxicity issues

1080-induced diabetes

Early reports (Cole et al 1955; Bowman 1964) noted that high acute doses of 1080 appeared to produce metabolic effects similar to those of diabetes (raised blood glucose levels), and the mechanism for this, at that time was unknown. This gave rise to the term "1080-induced diabetes". Cole et al (1955) investigated the possibility that 1080 exerts a target organ effect on the pancreas (effecting insulin-releasing cells) as a cause of this "1080-induced diabetes". They demonstrated that animals that recover from the acute effects of 1080, go through a period of raised serum glucose and ketosis, but subsequently recover with no sign of a permanent induction of diabetes from their acute exposure to 1080. They concluded that the "1080-induced diabetes" resulted from the effect of the fluorocitrate on the Kreb's cycle, not due to target organ effect of 1080 on the pancreas, which may have produced a permanent diabetic condition. The increase in serum glucose has been confirmed in more recent studies (Bosakowski and Levin 1986), although one study reported hypoglycemia (Iwasagi et al 1970). Findings reminiscent of diabetes have also been found in sheep (O'Connor et al 1999).

In the light of current knowledge, the apparent diabetes is the homeostatic response of the body to maintain cellular energy levels under the metabolic interference caused by fluorocitrate. Reduced phosphorylation of glucose is one likely cause of the raised serum glucose, while reduced cellular energy (ATP) levels may be involved (Bowman 1964). Pathways responsible for the increase in glucose levels such as phosphofructokinase (PKF) are likely to be involved, but the relative role of citrate inhibition of PFK in producing the rise in serum glucose is unclear (Godoy et al 1974; Stewart et al 1969).

The Agency notes that reference to the term "1080-induced diabetes" may give risk to misunderstandings about the impact of 1080 exposure. The Agency notes that the use of the term is of historical significance only. It

related to theories for the 1080 mechanism of action prior to the current level of biochemical understanding of this (even though this knowledge remains incomplete as noted above).

Mechanism for convulsions

Early reports assumed that the convulsions caused by 1080 were of central nervous system origin (Chenoweth 1949). More recently, Bosakowski and Levin (1986) concluded the mechanism of the tremor, tetany and convulsions in rats was reduced serum calcium level due to chelation with citrate. In dogs treated with lower doses (0.1 mg/kg bw), the increase in citrate is less pronounced, and heart ATP was not significantly reduced. Nevertheless, serum citrate correlated well with heart (cellular citrate), elevated serum glucose and reduced serum calcium. Serious clinical signs (convulsions) were associated with high serum citrate concentrations (which are in turn associated with low serum calcium levels). These findings are consistent with earlier work (Roy et al 1980). The authors emphasised that calcium citrate forms a stable, soluble complex so it is important to consider free calcium in serum rather than total calcium when assessing serum calcium levels.

Repeat dose tolerance

Repeat dose tests in rats and mice showed that tolerance to 1080 developed, if high sub-lethal doses were followed 24–48 hours later with even higher doses (Chenoweth 1949). Other early studies demonstrated that no protective effects of recent previous exposures occurred in sheep, indeed, suggested rather the reverse, that sheep were particularly sensitive to a serious of doses (Annison et al, 1960).

The Agency notes that since the protective effect relates to high doses of the chemical only, the tolerance, if found to occur in species other than in rats, is of no practical significance. This is particularly so, as the protective effect lasts only for about 48 hours after the earlier sub-lethal dose.

Latency period

An important characteristic of 1080 poisoning is there is a relative stable, symptomless, latency period before the relatively rapid onset of serious toxic signs and symptoms. This applies irrespective of route of administration (including, in particular, injection) so it cannot be related to the rate of absorption. The latency period is not greatly reduced by increasing the dose. This is in contrast with what would commonly be the case for other acute poisons. A latency period also applies following acute exposure in human (see section B17.1).

Explanations advanced to explain the latency period, have primarily focused on the need for metabolism of a critical quantity of the 1080 to fluorocitrate or for distribution of 1080 or its metabolites to critical targets (Chenoweth 1949). It has also been suggested there is a further delay

while there is the subsequent build up of fluorocitrate in the target organs before the toxic effects are manifest.

In a recent review, Clarke (1991) discussed the mechanism giving rise to the latency. In mice, intrathecal injection (an injection into the space around the spinal column) of fluorocitrate caused seizures in 15 seconds. In contrast, intracerebroventricular injection of fluoroacetate or fluorocitrate in mice caused seizures in about 30 minutes. It was also reported that co-administration of calcium attenuated the seizures. Clarke proposed that the latency period could be attributed to the time required for diffusion down the spinal column of the metabolites.

Toxicological significance of fluoride released from 1080

Early studies considered that the fluoride content of 1080 might be significant in relation to its toxicity (Chenoweth and Gilman 1946), but it was clear at an early stage that 1080 acts by a specific mechanism as discussed above, and the toxicity was unrelated to that of the fluoride content. Since this issue has been raised by some submitters the following material is included to indicate that the amount of fluoride in 1080 is practically toxicologically insignificant in comparison with the toxicity of 1080 itself.

Fluorine (atomic weight 19) represents 19% of the formula weight of sodium fluoroacetate (1080), formula weight 100. The minimum lethal dose of 1080 in humans is about 0.7 mg/kg bw. If the fluoride was all released from the 1080 the dose would be 0.133 mg/kg bw.

The estimated LD_{50} in humans of sodium fluoride is 70–140 mg/kg bw for an adult male (Hazardous Substances Data Base, 2007). Clearly, it is the fluoride that is toxic component in sodium fluoride. Taking the lower end of the range and correcting for the sodium composition, the toxic dose of the fluoride ion is approximately 70 * 19/(19 + 23) = 32 mg/kg bw.

A toxic dose of 1080 (0.7 mg/kg bw), would contribute only 0.133 mg/kg bw of fluoride (if it was all released a fluoride), so the amount of fluoride contained in a likely fatal dose of 1080 is approximately a 200th of that need to reach a toxic dose, with respect to the fluoride it contains. Since this is starting with the minimum **lethal** dose of 1080, it is clear that the fluoride content is not of toxicological significance, in comparison to the 1080.

B16.2 Mechanism of sub-chronic toxicity

The Agency found no general explanation in the scientific literature for the chronic and reproductive effects of 1080, so this is discussed in the following sections.

In relation to the heart, the assumption has been that longer term damage results from damage from the acute exposure to sub-lethal doses. This possibility has been supported by studies of acute doses in sheep

(Wickstrom et al 1997). The small foci of damage were found, and while of questionable toxicological significance according to the authors, is seems likely they had been caused by target organ effects on heart cells from the acute exposures resulting in death of some cells followed by repair processes. This is supported by the observation the sub-chronic doses at which effects are seen, represent a significant proportion of the acutely toxic dose. Nevertheless this is an unproven mechanism and remains speculative.

It seems likely that a similar mechanism may be responsible for the long-term damage found in the central nervous system (CNS) in one human case (Trabes et al 1983) discussed in section B17. The Agency notes that toxic effects on the CNS from equivalent longer term sub-chronic exposures have not been demonstrated in animal studies, but the extent to which such effects would be detected in sub-chronic animal tests is uncertain. Although the sub-chronic studies were aimed particularly at detecting male and female reproductive effects, at least one study reported that no adverse behavioural findings were found (Eason and Turck 2002).

B16.3 Mechanism of reproductive toxicity

The mechanisms of action for the developmental and reproductive toxicity findings are discussed separately.

B16.3.1 Developmental effects

Developmental toxicants generally target rapidly developing areas of the embryo and act either by means of mutagenic activity on the dividing cells (not considered likely for 1080 as discussed below) or by interference in the metabolically active cells. Selective interference with the proliferation, growth and development and scheduled death of cells in critical structures may be involved. These effects are often very time-dependent. With a metabolic poison such as 1080, such a mechanism seems likely. Although some investigation of such mechanisms were attempted in early reports these were not conclusive.

The Agency notes that ossification defects (abnormal development of skeletal structures and bone) could be related to citrate build-up, as this may interfere with calcium availability. While this is a plausible explanation for ossification defects, the Agency notes that ossification defects are quite often encountered as a result of toxic effects without the precise cause being determined and without any known disruption of calcium distribution.

The Agency notes that in relation to the developmental effects of 1080 that the only species in which these have been identified is the rat. The availability of data for other species would give some indication of whether or not the effect is species specific and the degree of variation in sensitivity between species (see section B13.2).

B16.3.2 Endocrine disruption

The possibility of an endocrine disrupting activity by 1080 was raised by Weaver (2003) apparently on the basis of the demonstrated reproductive and developmental toxicity findings, rather than any demonstrated ability to bind to endocrine receptors.

The possibility of the reproductive (developmental) effects being the result of endocrine disrupting activity was considered by Tremblay, Fisher and Leusch (2002). The purpose of the study was to determine whether or not 1080 or fluorocitrate, the major metabolite of 1080, bind to the ligand-binding domain of the mammalian oestrogen receptor. The study used a competitive binding *in vitro* test system to determine the concentration needed to displace labelled oestrogen, in comparison with the findings for various compounds known to give a positive response (o,p-DDT and nonyl phenol). Tamoxifen (an anti-oestrogen pharmaceutical agent) was also used as a positive control. The conclusion was that neither 1080 nor fluorocitrate are likely to promote oestrogenic or anti-oestrogenic effects by binding to the mammalian oestrogen receptor.

While this study did not indicate 1080 is likely to have endocrine disrupting activity, only one of the potential endocrine disruptor binding sites has been examined. Androgenic hormone binding sites were not tested; nor were more advanced tests carried out in which oestrogen-binding using cultured cells or entire animals done.

In a subsequent, more advanced, study Tremblay et al (2005) demonstrated that neither fluoroacetate nor fluorocitrate bind competitively to both androgen and oestrogen receptors to a significant degree, nor do they exert either agonistic or antagonistic effects on the binding of the physiological agonist. There is a very slight amount of uncertainty in this work in respect to the proportion of the fluorocitrate which would be the (-) erythrofluorocitrate, the toxic isomer produced enzymatically from fluoroacetate. Nevertheless, given the concentrations in the mixture used, the results indicate no effect of the “active” isomer.

Weaver (2003, 2006), claims there to be an endocrine disrupting effect, and appears to reach this conclusion on the basis that reproductive and/or developmental effects have been demonstrated. The Agency considers there needs also to be evidence that these effects are mediated by interference in the interaction of endocrine hormones and receptors, before it could be concluded that endocrine disruption is involved. The tests that have been done do not show any activity via endocrine receptors. There are numerous other mechanisms by which the reproductive and developmental effects may be caused. The Agency recognises that the mechanism for these effects remains unknown, but concludes that there is evidence showing 1080 is not operating via endocrine disruption.

B16.3.3 Reproductive toxicity: Sub-chronic effects on the testes

The sub-chronic effects on the testes remain unexplained. Early studies into the high sensitivity of testes to 1080 focused on the aerobic respiration and attempted an explanation relating to biochemical differences in the testes. The Agency did not identify any more recent studies that attempted to clarify the mechanism. Perhaps it is significant that that, at least in mice, the testes were found to be the organ least able to break the carbon-fluorine bond in fluoroacetate (apart from the brain) (see section B15.2.2) (Soiefer and Kostyniak 1983).

A recent study investigated both the mechanism of cell death in the testes after 1080 exposure (Shinoda et al, 2000). Male Sprague Dawley rats were given a single dose of 0.5 or 1.0 mg/kg bw of 1080 by gavage. The testes were examined from three rats at 3, 6, 12, 24, and 36 hours. No morphological changes occurred at any time following exposure at the lower dose rate. At the higher dose levels the researchers found that 1080 caused both rapid necrosis from depletion of cellular energy stores in spermatids and apoptosis in spermatogonia, but at later time points fluoroacetate inhibited spontaneous apoptosis of spermatogonia. The study clearly demonstrates a rapid effect on germ cells in the testes.

The key aspect relating to reversibility of the effects of 1080 is whether in addition to these differentiated cells the 1080 is toxic to spermatogonia (spermatoblast) cells that line the seminiferous tubules and maintaining the stem cell population for the process of spermatogenesis. Alternatively, 1080 may be causing its effect by disrupting the supporting sertoli cells that nourish and maintain the reproductive cells.

The Agency concludes that whether the testicular toxicity of 1080 is reversible is a matter that has not yet been resolved.

B17 Human poisonings from 1080

B17.1 Human toxicity reports related to 1080

Information on the toxicity of 1080 to humans is not directly relevant to the class 6 toxicity classification, except for end points for which a classification based on expert judgement is provided for. Nevertheless, the information is important for the assessment of human health risk. Therefore, a review of human exposure and toxicity data, particularly, from acute exposures has been included here.

Human poisoning from 1080 has rarely occurred in New Zealand. One contributing factor to this may be the extensive controls in place on the substance, limiting availability to the poison. Data relating to recent New Zealand poisoning cases (and enquiries relating to 1080 generally) are discussed in section M4.1.4 of Appendix M.

The 1080 guidelines (Department of Labour 2002) report that the National Poisons Centre was aware of two earlier cases of 1080 poisoning (before

the date range covered in Appendix M4.1.4). The second case appeared to be a fatality due to 1080, but whether this was accidental or the result of deliberate ingestion (suicide) is not stated. It does appear to be a confirmed fatal case of 1080 poisoning in New Zealand.

There was an earlier report of 1080 poisoning in New Zealand in the literature. The report (Parkin et al, 1977) claimed a case of chronic 1080 poisoning in an occupationally exposed rabbiter. The paper's interpretation of the associated analytical results generated considerable editorial comment. On the basis of the subsequent debate and the associated uncertainties, the Agency concluded that the study cannot be considered to be proven, so it does not form a reliable basis for regulatory decision making.

There have been quite a number of reports of acute poisoning from 1080 use overseas, since it was introduced as a rodenticide. These are clinical reports of the toxic effects, treatments and the resulting outcomes after human exposure to the substance (Chi et al, 1996; Gajdusek and Luther 1950; Trabes et al 1983).

The most common cause of 1080 poisoning appears to be deliberate consumption of substances containing 1080 with suicidal intent (Trabes et al 1983). The second most likely cause is accidental poisonings of young children. Most accidental poisonings have resulted from inappropriate use of 1080 around the home for rodent control or from inappropriate storage of surplus material (Gajdusek and Luther 1950; Hojer et al 2003).

The reports of human poisoning demonstrate that with respect to acute toxicity of 1080, the human signs and symptoms are essentially the same as those of animals. There is a symptom-free period, followed by a rapid onset of severe poisoning symptoms. Seizures, ventricular fibrillation are particular features, the latter in particular appearing to indicate an adverse outcome.

Despite quite a large number of human poisoning cases, there are no clinical biochemical test results that can be used to give an indication of human toxicokinetics for 1080; nor do the studies give clear indications of minimum lethal dose in the human. This is probably not surprising as in the context of human poisonings the dose received is often very uncertain. If clinical toxicology investigations had been done, the data may give a more accurate indication of how much 1080 was actually consumed. That would be useful, as the basis for the estimates for human toxic or lethal doses of 1080 are not well documented.

Chi et al (1996) reviews the findings from 38 cases of human suicidal 1080 poisoning (seven of which were fatal) in an attempt to identify clinical and laboratory features associated with a fatal outcome (Chi et al 1996). Since this was a retrospective study, they could not address deficiencies in the record. Statistics for the whole group were not given, but judging from the age distribution for the survivors and fatalities most patients were adults. The average age for survivors was 40.8 years (SD 18.6), whereas for the

seven non-survivors (18%) the average age was 47.3 years. Hypotension, an early onset of metabolic acidosis and an increased in serum creatinine were associated with a poor prognosis (a fatal outcome). Reduced serum calcium was more common in non-survivors, but not to a statistically significant degree. The paper includes no estimates of 1080 doses the patients received, nor analytical findings relating to concentration of the poison or its metabolites in biological samples. Symptoms and signs seen included nausea, vomiting, abdominal pain, diarrhoea, anxiety, agitation, respiratory distress, muscle spasms, stupor, seizure and coma. Acute oliguric renal failure (renal failure associated with production of a low urinary volume) occurred in two non-survivors. Of the seven fatalities, only two patients suffered seizures. This proportion seems surprisingly low, and may reflect the partial effectiveness of therapeutic measures undertaken. No information is given on the condition of the surviving patients at time of discharge or follow-up.

Some clinical reports have made it clear that even after 1080 intake in humans sufficient to generate life-threatening symptoms, such as seizures, if adequate treatment is available, complete recovery is possible (Robinson et al 2002). In this instance, the 47-year-old male vomited 2 hours after ingestion, and at 34 hours was unresponsive except to noxious stimuli (indicating a deep coma), but although the patient suffered seizures, he was discharged asymptomatic 10 days after admission.

Similar outcomes are also possible in young children (Gajdusek and Luther 1950). In this latter case the 2-year-old boy survived apparently with no adverse effects. He had vomited extensively, but still suffered seizures. It was not until seizure commenced six hours after the ingestion of the poisoning that he was rushed to hospital. He suffered seizures and periods of coma. His heart rhythm became irregular and there were periods of ventricular fibrillation, alternating with periods of asystole or tachycardia (ceased or raising heart beat). While in retrospect the inappropriate treatments for fluoride and arsenic poisoning (despite the parents having brought in the bottle labelled “Rat poison–1080”) is noted, and possibly some symptom could have been adverse effects from the treatments, the report is remarkable for the length of time before recovery commenced, and then was very clear, on the fourth day after admission. The study reports that the boy was well at follow-up one year after discharge and there were no apparent effects relating to neurological of cardiac function.

Reigart et al (1975) gives an account of poisoning in a very young child (8 months), after she had been seen chewing on the cap left behind the refrigerator some months earlier. Despite a protracted course of treatment in the hospital during which time she suffered seizures and irregularities of heart rhythm, although this did not include fibrillation. The most significant aspect was that the first seizure did not occur until 20 hours after the ingestion of the poison. This paper is referred to by others (Robinson 2002) due to the remarkably long latency period. Although it would be unreliable to reach conclusions on the basis of one case, it may be that the latency period in humans is longer than in animal models. Such

a conclusion would be consistent with the proposed mechanism for the latency is discussed by Clarke (1991) (see section B16).

The report of the attempted suicidal poisoning by a young woman (Trabes et al 1983) is of importance as it is a rare example in which long-term adverse effects have occurred following an acute 1080 poisoning. After extensive therapeutic intervention, the 15-year-old girl suffered longer term brain damage, verified by a brain scan, following a single ingestion of 1080. The study does not provide information about the likely dose of 1080 ingested. It is likely that without the emergency treatment survival would have been impossible.

There is a report of complete recovery of several children even after serious toxic effects (Reigart et al 1975; Gajdusek and Luther 1950). After accidental poisonings the quantity of poison consumed is uncertain. The first case was unusual in several respects. Clear symptoms and signs indicative of 1080 poisoning (seizures) did not occur until about 20 hours after the poisoning. Earlier induction of emesis (twice) may have been crucial in the final outcome. In the second case, again the dose appears low, but effective first aid measures were less prompt and efficient.

A single case of 1080 poisoning from inhalation of 1080 is also of importance. In the first hand account of a single inhalation exposure to 1080 in the form of powder (Williams 1948), the symptoms and course were described. Exposure was to a small quantity of powdered sodium fluoroacetate, which is the technical grade active, while the person was weighing it for preparation of rodent baits. The report is particularly noteworthy because it represents the only inhalation-exposure documented in animals or humans. Also, the patient was medically trained, and able to give a first-hand account of the symptoms they experienced, as far as they could recall them.

Initially there was numbing of the face, a sour tart taste and tightness around the mouth and nasal passages. The whole face became numb and tingling extended down the limbs. Seizures then started, followed by loss of speech and eventually unconsciousness 2½ hours later. The length of time before the muscle spasms is unclear, but the account implies a relatively rapid onset of symptoms, although unconsciousness was delayed. While there is no indication of the dose received, the indication is of an exposure to a single “puff of the powder”, so a relatively low dose seems likely to have occurred. The study provides indirect and unconfirmed evidence that 1080 technical grade active is highly toxic by inhalation, as would be expected.

B17.2 Discussion

The Agency notes that the applicants claim in their assessment of possible effects on the general public, that no cases of accidental or deliberate poisoning from 1080 have been reported in New Zealand (H-A19, p241). In the review above, the Agency identified at least one (fatal) case, which

occurred in New Zealand, but the circumstances were not available (Department of Labour 2002).

The applicants also claim in the same section (H-A19) that provided the dose was sub-lethal, small quantities of 1080 can be metabolised (by humans) and/or excreted in the same manner as for the target pest. By implication, the applicants assume that such a situation would not result in any permanent harm. The Agency notes that such a conclusion is consistent with the general principles of toxicology for a non-symptomatic dose (except for the risk assessment of genotoxic carcinogens, which is not relevant here). Nevertheless, the Agency is not aware of good information relating to human doses that established lowest toxic doses, and by implication doses below that that are likely to be non-toxic. Estimates for human doses that are likely to be fatal are listed discussed in Appendix M4.1

As discussed in above, at least one human acute poisoning (from a single dose) has caused prolonged disability (Trabes, et al 1983), although this appeared to be after a fatal dose was taken and the patient was saved from death only by medical intervention. This does make it clear that an acute toxic dose, if survived, may be responsible for permanent harm.

B17.3 Conclusion

The Agency concludes that the above human data support the view that with respect to acute toxicity of 1080, effects on humans are similar to effects on other terrestrial mammalian species.

B18 Antidotes

B18.1 Data

The Agency came across a significant number of studies which attempted to identify and verify the validity of 1080 antidotes (Chenoweth and Gilman 1946) Some early reports identified monacetin, acetate and ethanol as potentially beneficial. While greater length of survival could be achieved in mice, rats, rabbits and guinea pigs with the use of acetate and ethanol, this was not successful for the most sensitive species (dogs), and thus its value was questionable (Tourtellette and Coon 1950). The lack of value of the antidotes was highlighted by the observation that while the treatment was beneficial in dogs when administered immediately, treatment would need to start before symptoms appear, within 30 minutes. Clearly this is not feasible in the field with dogs not known to have been poisoned.

In spite of these early reports the Agency has been advised by the National Poison Centre (Beasley, 2007, personal communication) that the Centre does not currently recommend any 1080-specific antidotes as being effective.

The Agency, nevertheless, found that based on literature reports, symptomatic treatments can be successful even in cases where serious toxic effects manifest. A number of such cases were discussed above (Robinson et al 2002; Gajdusek and Luther 1950).

Despite a relatively good understanding of the mode of action of 1080, little progress has been made in the development of treatment options. Treatment remains essentially symptomatic and the reliant on the control of crucial features such as the seizures and, the life-threatening conditions, such as cardiac arrhythmia and respiratory failure. Treatment of these life-threatening conditions require advanced medical intervention, so that only when individuals can be transported to a major medical facility is successful treatment after a toxic dose likely. More recent studies taking into account a more advanced understanding of the mechanism of action may offer options for development of antidotes. For example restoring serum calcium slowed the onset of seizures (Clarke 1991) extended survival in anaesthetised cats (Taitelman et al 1983).

B18.2 Agency conclusion

The Agency noted that the absence of specific 1080 antidotes is of significance for human risk assessment as poisoning with 1080 can occur accidentally. Nevertheless, symptomatic treatments (treatment of the patient with extensive intervention to address the toxic effects as they arise) have been found to be effects. Comparison with the situation with respect to cyanide is a point of difference as extensive, well proven, antidotes are available for cyanide (although the successful use of a selection of these in remote areas by poisoned individuals is less well documented). The difference in availability of antidotes is reflected slightly in the assessment of human health risks in Appendix M and section 7.4.

B19 Classification of the formulated products containing 1080

B19.1 Approach

All the human toxicity classifications for the 1080 formulations are driven by the 1080 content in the formulated products. The Agency agrees with the classifications proposed by the applicant for each of the formulated products. This section sets out the approach to the classification.

A change that was considered appropriate for the classification for one gel formulation in comparison with the previous classification (from the deemed “transfer” approval) and the basis for it, is discussed.

B19.2 Acute oral toxicity (6.1)

The classification for acute toxicity is carried out using the additivity formula. The LD_{50} value used for this calculation is the lowest LD_{50} in a

suitable laboratory species, which in this case 0.066 mg/kg bw from the dog. The additivity formula is:

$$\frac{100}{LD_{50}\text{ mix}} = \frac{\%\text{ Component A}}{LD_{50}\text{ Component A}} + \frac{\%\text{ Component B}}{LD_{50}\text{ Component B}} + \text{(etc)}$$

The equation becomes very simple for the 1080 mixtures because the only component triggering acute toxicity is the 1080. Therefore, the formula simplifies to the following:

$$\frac{100}{LD_{50}\text{ mix}} = \frac{\%\text{ 1080}}{LD_{50}\text{ 1080}}$$

Taking the substances "Paste containing 1.5 g/kg sodium fluoroacetate" as an example, the mixture contains 0.15% of 1080. Therefore, the calculation becomes:

$$\frac{100}{LD_{50}\text{ mix}} = \frac{\%\text{ 1080}}{LD_{50}\text{ for 1080}}$$

Rearranging the expression gives:

LD_{50} for the mixture = 100/(% of 1080/LD_{50} for 1080)

= 100/(0.15/0.066)

= 44 mg/kg bw

The resulting LD_{50} for the mixture is 44 mg/kg bw. This is compared with the cut-off values for oral toxicity set out in p 8 of Part VI of the *User Guide to Thresholds and Classifications under the HSNO Act* (available on the ERMA New Zealand website). The result falls in the range greater than 5 mg/kg bw and less than 50 mg/kg bw so the mixture is 6.1B for acute oral toxicity.

The equivalent calculation is done for the concentration of 1080 in each mixture and the result compared with the cut offs to give the resulting acute oral toxicity classification, the results are summarised in Table B8. (The relevant classification thresholds are included in a note to the table.)

Table B8: Summary of acute toxicity classifications for 1080-formulated products

Name of approved substance (g/kg)	Concentration of 1080 (%)	Calculated LD_{50} of the mixture (mg/kg bw)	Classification[1]
Pellets containing 0.4–0.8 g/kg 1080	0.04–0.08%	82.5–165	6.1C
Pellets containing 1.0 g/kg 1080	0.1%	66	6.1C
Pellets containing 1.5–2.0 g/kg 1080	0.15–0.2%	33–44	6.1B
Paste containing 0.6–0.8 g/kg 1080	0.06–0.08%	82.5–110	6.1C
Paste containing 1.5 g/kg 1080	0.15%	44	6.1B

Name of approved substance (g/kg)	Concentration of 1080 (%)	Calculated LD_{50} of the mixture (mg/kg bw)	Classification[1]
Paste containing 10 g/kg 1080	1.0%	6.6	6.1B
Gel containing 1.5 g/kg 1080	0.15%	44	6.1B
Gel containing 50 g/kg 1080	5%	1.32	6.1A
Gel containing 100 g/kg 1080	10%	0.66	6.1A
Stock solution	20%	0.33	6.1A

Note

1 The thresholds for the relevant classifications are as following ranges (taken from *User Guide to Thresholds and Classifications under the HSNO Act* (ERMA New Zealand 2001):

6.1A oral LD_{50} below 5 mg/kg bw

6.1B oral LD_{50} above 5 mg/kg bw but less than 50 mg/kg bw

6.1C oral LD_{50} above 50 mg/kg bw but less than 300 mg/kg bw.

B19.3 Dermal toxicity (6.1)

The equivalent additivity calculations for the formulations can also be done based on the dermal LD_{50} value. However, the oral classifications will always be higher than that for the dermal route, so the oral classification will drive the overall classification. Therefore, the calculations and resulting dermal classifications have not been documented here.

B19.4 Inhalation (6.1)

See also the discussion under the acute inhalation classification of the active ingredient above.

The Agency considers section 77A of the HSNO Act should be used to apply the controls that would apply if the appropriate inhalation classification applied to 1080 mixtures.

The Agency considers that it is appropriate to apply an inhalation classification to:

- solid formulations of 1080 (pellets) because they may give rise to dust containing 1080 during storage and handling
- stock solution containing 20% 1080 because it has the potential to generate a mist when sprayed.

No inhalation classification is proposed for paste or gel baits containing 1080 as they are unlikely to generate dusts or mists.

In the absence of an LC_{50} value for 1080, the question remains how the classification of the formulations can be derived. The Agency concludes the only basis for a decision was expert judgment and recommends the following classification:

Pellet baits
Since the concentration of 1080 in these baits is less than 0.2%, the Agency considers the toxicity is relatively low, and proposes 6.1C.

Stock solution
Since the stock solution contains 20% of 1080, the Agency considers the toxicity is high, so proposes 6.1A to identify the magnitude of the hazard.

- ***Classification: Pellet baits: Not classified, but assigned controls equivalent to what would apply for 6.1C (inhalation)***

- ***Classification: Stock solution: Not classified, but assigned equivalent controls equivalent to what would apply for 6.1A (inhalation)***

B19.5 Skin irritancy (6.3)

The 1080 triggers classification as a 6.3B skin irritant. The mixture rules state that the mixture is assigned the 6.3B classification if it contains 10% or more of the triggering component. Therefore, 6.3B is triggered for mixtures containing 10% or more of 1080. Therefore, only the Gel containing 100g/kg and the stock solution are classified 6.3B skin irritants.

B19.6 Eye irritancy (6.4)

The 1080 triggers classification as a 6.4A eye irritant. The mixture rules state that the mixture is assigned the 6.4A classification if it contains 10% or more of the triggering component. Therefore, 6.4A is triggered for mixtures containing 10% or more of 1080. Therefore, only the gel containing 100g/kg and the stock solution are classified 6.4A, eye irritants.

B19.7 Respiratory sensitisation (6.5A)

No components in any of the formulations trigger this hazard.

B19.8 Contact sensitisation (6.5B)

The classification of one substance, "Gel containing 1.5 g/kg sodium fluoroacetate", by the applicants is different from that applied by the Agency in the Hazardous Substances (Sodium fluoroacetate) Transfer Notice 2005. "Gel containing 1.5 g/kg sodium fluoroacetate" was classified as a contact sensitiser (6.5B) in that notice.

This classification has been removed. The mixture contains a substance that has been classified as a contact sensitiser, 6.5B classification. While the Agency still considers the component is a contact sensitiser the Agency considers that it is not appropriate for this classification to apply to the mixture, due to the low concentration present in the gel, "Gel containing 1.5 g/kg sodium fluoroacetate".

Therefore, the Agency agrees with the applicant that the 6.5B classification should be removed for this substance. The effect of this will be that "Gel containing 1.5 g/kg sodium fluoroacetate" and "Paste containing 1.5 g/kg sodium fluoroacetate" will have the same classification.

B19.9 Mutagenicity (6.6)

No components in any of the formulations trigger this hazard.

B19.10 Carcinogenicity (6.7)

No components in any of the formulations trigger this hazard.

B19.11 Reproductive and developmental toxicity (6.8)

1080 triggers the 6.8A classification, and this classification is triggered for mixtures containing 0.1% or more of 1080.

Note that the application states (incorrectly) on p 100 that the 6.8A applies to formulations at 1% or above. The correct cut-off concentration is 0.1% as the application states under Section 4.7.5 on p 102 of the application. Despite the wording error, the applicant used the correct value when assigning classifications so they are correct. All the formulated products trigger this classification except for the two lowest pellet and paste formulations.

B19.12 Target organ systemic toxicity (6.9)

1080 triggers the 6.9A classification. The classification of mixtures for this end point is slightly more complex. The classification 6.9A is triggered for mixtures containing 10% or more of 1080. The classification 6.9B is triggered for mixtures containing 1% or more of 1080 (but not more than 10%) of 1080.