ASSESSMENT OF POTENTIAL RISK TO HUMAN HEALTH FOLLOWING USE OF AZAMETHIPHOS, DELTAMETHRIN AND HYDROGEN PEROXIDE IN FISH FARMS

REPORT TO SCOTTISH SALMON PRODUCERS ORGANISATION FROM WCA

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EXECUTIVE SUMMARY

The objective of this project was to assess the potential health risk to open-water swimmers in the vicinity of fish farms in Scotland in relation to medicinal treatments applied for the control of sea lice on salmon. The three substances assessed were azamethiphos, deltamethrin and hydrogen peroxide; these substances forming the active ingredients of products licensed for medicinal use on fish farms. Medicinal sea lice treatments using known amount of the substances are carried out in one of two ways:

- Bath treatments in-situ. By enclosing the pen in question fully with a large tarpaulin. The net is lifted to gently crowd the fish together in the smallest safe volume. The tarpaulin is passed underneath the net and pulled up around the pen above the water level. When the fish are totally enclosed in the tarpaulin, treatment can begin. Once the treatment is completed the tarpaulin is removed and the treatment water released into the sea.

- Fish may be treated in tanks on board specialist wellboats. Following treatment, the dislodged lice are collected and disposed of, then the treatment water is released into the sea.

The procedure followed in the current assessment can be summarised as follows:

1. Detailed literature searches, including "grey literature", for relevant toxicological safety data for the substances. The toxicological data searched for included all relevant exposure routes (dermal, inhalation and oral) together with the relevant areas of toxicity (mutagenicity, carcinogenicity, repeat-dose toxicity, reproductive toxicity);

2. Review of toxicology data and identification of relevant No Observed Adverse Effect Levels (NOAELs) in toxicology studies to identify points of departure (PoDs), from which Derived No Effect Levels (DNELs) for the human population are determined;

3. Derivation of relevant DNELs for oral and dermal routes of exposure following the relevant guidance documents. The major routes of exposure include oral and dermal exposure. Inhalation and respiratory exposure are considered minor routes of systemic exposure to swimmers, therefore, inhalation exposure was evaluated qualitatively according to the local toxicity of the substances, and appropriate adjustments made to the assessment factors for DNEL calculations;

4. Risk characterisation; this consisted of deriving predicted swim-water concentration for each treatment substance based upon derived safe exposure levels which, when not exceeded, could be deemed safe for open-water swimmers. This level was then compared to the concentration used in the treatment bath to calculate a risk characterisation ratio (RCR) for each substance.

The predicted swim-water concentrations are derived based on a number of worst case assumptions for a standard 71.8 Kg adult human:

- That the water concentration of the substance is constant irrespective of environmental conditions e.g. temperature, wind, water flow etc.;
- That the water concentration is constant irrespective of treatment frequency;
- That the swimmer is moving through a static plume, with no adjustment for distance from farm or distance travelled while swimming;
- No allowance for residue degradation or dilution of the substances in the water;
- 100% absorption by dermal and oral routes of exposure;
- No allowance for metabolism or excretion;
- A 2 hour swim with no protection worn (e.g. wet suit);
- Application of standards based on SWIMODEL data (US EPA 2003).

The relevant data and assessment outputs for each of the three substances are summarised in the following Table.

Summary of risk assessment data

Data	Information	Azamethiphos	Deltamethrin	Hydrogen peroxide
Source studies	Oral	90-day oral gavage repeat-dose neurotoxicity study in rats (2009)	90-day oral gavage repeat-dose toxicity study in rats (1977)	100-day oral gavage repeat-dose toxicity in rats (1969)
	Dermal			
Point of Departure (PoD)	Oral	0.05 mg/kg/day	1 mg/kg/day	20 mg/kg/day
	Dermal			
DNEL	Oral (mg/kg/day)	0.00125	0.025	0.5
	Dermal (mg/kg/day)	0.0025	0.05	0.33
SWIMODEL water concentrations (mg/L)	Oral	0.150	2.99	59.84
	Dermal	0.411	8.22	54.24
	Lowest	0.150	2.99	54.24
Maximum concentration used to treat fish (mg/L)		0.12	0.002	1500
Risk characterisation ratio (RCR)		0.8	0.0007	27.7

The risk characterisation ratios for azamethiphos and deltamethrin were determined to be 0.8 and 0.0007, respectively. As these values were both below 1, it can be concluded that the concentrations of azamethiphos and deltamethrin used to treat fish are below the concentrations predicted by SWIMODEL to present no hazard to swimmers (on a worst-case basis). This demonstrates that the concentrations used to treat fish are safe for open-water swimmers, even before dilution and dispersion occurs in open waters. However, for hydrogen peroxide, the risk characterisation ratio was determined to be 27.7. As this value is above 1,

this indicates a risk associated with the concentrations of hydrogen peroxide used in the fish treatment baths. Therefore, characterisation of dilution and dispersion factors are likely to be required to be taken into account to demonstrate that discharges of hydrogen peroxide are safe for open-water swimmers[1].

[1] An assessment of such dilution and dispersion characteristics for hydrogen peroxide (undertaken by Salmon Scotland) is given in Appendix 1.

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TABLES

1 INTRODUCTION

The Scottish Salmon Producers Organisation (SSPO) requested wca undertake an investigation into the potential risk to humans, specifically open-water swimmers in the vicinity of fish farms in Scotland, of the organophosphate pesticide, azamethiphos (CAS# 35575-96-3; EC# 252-626-0), the pyrethroid ester pesticide, deltamethrin (CAS# 52918-63-5; EC# 258256-6) and the reactive oxygen species chemical, hydrogen peroxide (CAS# 7722-84-1; EC# 231-765-0), used in the routine control of external parasites, namely sea lice of farmed salmon.

Medicinal sea lice treatments using known amount of the substances concerned are carried out in one of two ways:

- Bath treatments in-situ. By enclosing the pen in question fully with a large tarpaulin. The net is lifted to gently crowd the fish together in the smallest safe volume. The tarpaulin is passed underneath the net and pulled up around the pen above the water level. When the fish are totally enclosed in the tarpaulin, treatment can begin. Once the treatment is completed the tarpaulin is removed and the treatment water released into the sea.

- Fish may be treated in tanks on board specialist wellboats. Following treatment, the dislodged lice are collected and disposed of, then the treatment water is released into the sea.

The objective of this project was to produce a report containing a summary of the toxicological profile of the three substances, together with potential derived No effect levels (DNELs), using standardised methodology. In addition, safe exposure concentrations in water have been predicted, based on a suitable model (US EPA Swimmer Exposure Assessment Model (SWIMODEL)). These safe exposure concentrations were then compared with the concentrations of treatment-bath medicines applied to the salmon, before being discharged into the sea.

This process is based on a number of worst-case assumptions, including that the water concentration is static and equal to the concentration used to treat salmon (prior to discharge), irrespective of environmental conditions, treatment frequency and residue degradation/dilution; 100% absorption of the substance is via the oral and dermal routes of exposure; and that each swim is of a 2 hour duration, with no protection worn (e.g. wet suit).

2 METHODS

Firstly, a toxicological database was established including all available regulatory information. Detailed literature searches were conducted to identify sources of relevant published toxicological data. The assessment of all relevant toxicological safety data revealed suitable points of departure such as No Observed Adverse Effect Levels (NOAELs), No Observed Effect Levels (NOELs) or Low Observed Adverse Effect Levels (LOAELs) from which Derived No Effect Levels (DNELs) were derived.

Azamethiphos is not a registered chemical under the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), however, there is a Harmonised Classification and Labelling (CLH) report available which was submitted in 2018. Sufficient toxicological data was available in this report to support this assessment, although a literature search was also carried out to identify other potential sources of relevant information, such as regulatory submissions under Plant Protection Product (PPP) regulations.

Deltamethrin is not a registered chemical under REACH, however, it is a registered plant protection product therefore a Draft Renewal Assessment Report for Deltamethrin was available through the European Food Standards Agency (EFSA) (EC 2017). Sufficient toxicological data was available in this report to support this assessment, and the relevant data were extracted from this report.

Hydrogen peroxide is a registered chemical under REACH, therefore, relevant toxicological data is available from the European Chemicals Agency (ECHA) registration dossier. Additionally, a Cosmetics Ingredient Review was conducted for hydrogen peroxide in 2018, and relevant toxicological data were also available from this report.

Given the amount of information available on the substances of interest, at this stage it was considered that identification of potential read-across substances for the three substances under investigation would not be necessary.

2.1 Review of toxicological data

All relevant toxicological data for the target substances were reviewed and used to identify appropriate Points of Departure (PoDs) from which to derive safe exposure levels.

2.1.1 Literature search and review

The toxicological databases were searched over a specific time period, as identified in the substance specific sections, for relevant data pertaining to hazards and safety of each compound (toxicological effects, metabolic fate and toxicokinetics) using appropriate search strings. The resultant identified references (titles, authors, abstracts etc.) were incorporated into an Excel spreadsheet format and reviewed for relevance. A list of additional references considered necessary for the completion of the assessment was compiled and the references obtained.

Searches of the open scientific literature were conducted using the following literature databases:

- Web of Science[2] (from Clarivate Analytics) – A bibliographic database covering scientific literature from the Web of Science™ Core Collection, including BIOSIS Citation Index, BIOSIS Previews, Biological Abstracts, Zoological Record, MEDLINE, CAB Abstracts, CABI Global Health, Inspec and Data Citation Index.

- PubMed[3] (from the US National Library of Medicine) – A bibliographic database providing comprehensive coverage of the biochemical, pharmacological, physiological, and toxicological effects of drugs and other chemicals. The database includes scientific literature from PubMed/MEDLINE, DART, NTIS, RePORTERTOX, as well as from several other archival collections.

Each database was searched initially with two sets of keywords i) terms for the target and source substances (e.g. chemical name, synonyms, CAS number) and ii) relevant human health and toxicology search terms. The dates that the databases were interrogated were recorded.

The resulting references from the searches were downloaded into an Excel spreadsheet as a record of the searches. The titles and abstracts of the articles were screened for potentially relevant articles, although in some cases the initial review focused on titles only, in order to maximise the time spent on reference selection. Selected references were copied to a separate tab. These were cross-referenced with the human health studies reported in secondary literature sources and any previous reviews of each of the target and source substances. The full search record and selected references recommended for review were then shared with the client. Following agreement of these references with the client, the full papers were obtained and reviewed for the project.

However, it is important to note that the academic literature may be biased towards novel, non-GLP technologies, neglecting standardised and validated test reports (e.g. GLP-compliant OECD TG studies). Consequently, the data mining of 'grey literature' was also performed. The European Commission (EC) Competent Authority for REACH and CLP (CARACAL) meeting reports, EFSA, Chemical Watch and ECHA websites were reviewed for further information. This additional step not only identifies proprietary data but also provides a broad overview for the identification of other potentially relevant academic studies, via cited references. Secondary data were cross-referenced with the studies obtained in the systematic literature search.

2.1.2 Method of Reliability and Quality evaluation of Data

The reliability of all published *in vivo* mammalian toxicity studies from which dose levels have been selected as PoD are assessed using the software-based Toxicological Data Reliability

[2] https://wok.mimas.ac.uk/
[3] https://www.ncbi.nlm.nih.gov/pubmed

Assessment Tool ('ToxRTool'). The ToxRTool evaluation method is an xls-based toxicological data reliability assessment tool and provides comprehensive criteria and guidance for evaluations of the quality of toxicological data. The ToxRTool leads to the assignment of Klimisch categories 1, 2, or 3 (Klimisch 1997) in a transparent and robust manner. The Klimisch scoring system is as follows:

> **1. Reliable without restriction**: data from studies carried out to nationally or internationally accepted guidelines, preferably performed to GLP, or where all parameters described are comparable to guideline methods.
>
> **2. Reliable with restrictions**: data from studies which do not completely follow the test guideline, and which may not be performed to GLP, but which are sufficiently well documented and scientifically acceptable.
>
> **3. Not reliable**: data from studies with interferences between the test substance and measuring system, or where the organism or test system is not relevant to the exposure. The methods used may not be acceptable, with insufficient documentation to allow expert judgement.

However, the majority of the toxicological data reviewed in this report are from summary documents or databases, such as the Harmonised Classification and Labelling (CLH) report for Azamethiphos (EC 2018), the Draft Renewal Assessment Report for Deltamethrin (EC 2017) and the ECHA dissemination portal for hydrogen peroxide. In these cases, the data cannot be assessed for reliability as the full study reports are not available, and therefore, we have defaulted to the Klimisch score assessments provided by the authors of these reports. Where available, these Klimisch scores are indicated in the tables of toxicological data for the substances.

2.1.3 Toxicological review

All the data gathered on the toxicological profile of the target substances including acute and repeat-dose toxicity, genotoxicity, carcinogenicity and reproductive toxicity were reviewed, and summarised in this report.

The objective of the subsequent systematic review of toxicity data was to identify suitable No Observed Adverse Effect Levels (NOAELs) or No Observed Effect Levels (NOELs) for the identified substances by all appropriate routes of exposure, where possible, including dermal, oral and inhalation. NOAELs and NOELs were identified in appropriate repeat-dose toxicity studies as being representative of the previously described exposure scenario. The selected PoDs were subsequently modified, where necessary, by application of Assessment Factors (see Section 2.2) to accommodate any further qualitative toxicological influences such as local irritation. All relevant PoDs for each of the substances contributing to this evaluation are presented in this report together with all relevant modifications for the route of exposure where necessary.

2.2 Calculation of Derived No Effect Levels (DNELs)

The methods for deriving no effect levels for the human population followed those described in ECHA Guidance[4]. The objective was to derive systemic DNELs for oral and dermal exposures for the General Population from the PoDs characterised by the dose descriptors, NOEL, NOAEL or LOAEL in the most relevant animal study(ies) for the target substance. A number of standardised Assessment Factors (AF) are applied to the PoD to extrapolate toxicity to the human population. It may also be necessary to make appropriate mathematical conversions of PoD information to express the DNEL in terms of mg/kg bwt/day. The inhalation toxicity data was included in the acute and local toxicity data assessment on the basis that this was considered a minor route of exposure (see Section 3).

2.3 Risk assessment

In order to undertake the risk assessment for open-water swimmers in the vicinity of a fish farm, it would be necessary to generate an estimation of the potential human exposure to each of the target substances represented by a residual concentration of the target substance in the waterbody used by the swimmers.

Inhalation and respiratory exposure are considered minor routes of systemic exposure to swimmers, therefore, inhalation exposure was evaluated according to the local toxicity of the substances. The potential inhalation exposure is considered of minor significance as a route of systemic absorption due to the lack of intention to inhale the water by the swimmer and the tussive response of the swimmer.

From the estimated human exposure level, quantification of exposure to recreational swimmers would consider the intrinsic chemical hazard, the exposure scenario and the possible exposure routes. The European Chemicals Agency (ECHA)[3] and US EPA[5] provide guidance documents for exposure quantification[6], highlighting equations to quantify exposure via skin contact and ingestion.

- Quantification of dermal exposure – the ECHA approach assumes that 100% of the substance is taken up by the skin independent of the exposure duration. Under these conditions, dermal uptake is dependent on the contaminant concentration in water C_{water}, the affected skin area and the event frequency (ECHA 2008a).
    - $E_{dermal} = C_{water}$ *x skin area x frequency/bw*

[4] ECHA Guidance on information requirements and chemical safety assessment. Chapter R8: Characterisation of dose (concentration)-response for human health. Version2.1. November 2012
[5] US EPA Swimmer Exposure Assessment Model (SWIMODEL) Version 3.0 (2003)
[6] ECHA Guidance on Information Requirements and Chemical Safety assessment. Chapter R7a: Endpoint Specific guidance. Version 6.0. July 2017

- Quantification of oral exposure – oral exposure is dependent on the contaminant concentration in source, the amount of ingested source, the event frequency and the bioavailability (ECHA 2008a)
    - $E_{oral} = C_{source}$ *x amount x frequency x bioavailability/bw*

However, the equations from the US EPA Swimmer Exposure Assessment Model[4] are considered more applicable to this "wild-swimming" scenario and are detailed below:

Oral exposure

A certain amount of water will inevitably be swallowed during a swimming session but oral ingestion is considered to be the intermediate mode of exposure and will be dependent on the estimated volume of contaminated water that is swallowed. Quantification of oral exposure is determined through the following equation:

$$PDR_{oral} = ET \times IR \times C_w$$

PDR: Potential dose rate via oral exposure (mg/swim)
IR: Ingestion rate
ET: Exposure time (hours/swim)
C_w: Concentration in water (mg/L)

Dermal exposure

Absorption of the chemical via the skin will be dependent on the surface area exposed and the Permeability Coefficient (Kp – cm/hr). Assuming that no protection against absorption is offered by the wearing of protective clothing (wet suit) then the dermal route is likely to represent the greatest potential route of exposure during a swimming session. Quantification of dermal exposure is determined through the following equation:

$$PDR_{dermal} = ET \times SA \times C_w \times K_p$$

PDR: Potential dose rate via dermal exposure (mg/swim)
ET: Exposure time (hours/swim)
SA: Skin surface area (m^2)
C_w: Concentration in water (mg/L)
K_p: Chemical specific permeability coefficient

Calculated or estimated human exposures to azamethiphos, deltamethrin and hydrogen peroxide are required for comparison with the derived no-effect levels (DNEL) calculated previously in order to generate Risk Characterisation Ratios (RCR) and Margins of Safety (MoS). The principal of the formulae detailed above, together with the procedural standards detailed in the US EPA Swimmer Exposure Assessment Model[4], have been used in combination to determine a hypothetical overall safe exposure to an open-water swimmer. However, in the absence of specific data relating to water concentrations in the vicinity of open-water swimmers, these can only be compared with the actual concentrations of the substances used to treat fish in a worst-case assessment. This process is detailed in Section 4.

3 TOXICOLOGICAL EVALUATION

For each substance, the toxicological evaluation summarises data available and derives DNELs for the primary routes of exposure: oral and dermal. The information for the substances azamethiphos, deltamethrin and hydrogen peroxide are presented in sections 3.1, 3.2 and 3.3, respectively.

3.1 Azamethiphos

3.1.1 Hazard assessment

3.1.1.1 Regulatory data

Azamethiphos is not a registered chemical under REACH, however, there is a Harmonised Classification and Labelling (CLH) report available which was submitted in 2018. Sufficient toxicological data was available in this report in order to complete this assessment, and relevant data was extracted from this report for azamethiphos.

3.1.1.2 Literature search

The search was conducted using the substance name and identifiers only and the results of the search are summarised in Table 3.1. Results were not date limited. No additional relevant toxicology information was found for the target substance.

Table 3.1 Azamethiphos - Literature search results

Search	Number of hits		Combined number of hits after duplicates removed
	Web of Science	PubMed	
(Azamethiphos)	91	158	185

Where references were identified, they were downloaded into an Excel spreadsheet as a record of the searches. The titles and abstracts of the articles were screened for potentially relevant articles. No relevant information was identified through the screening process, so no additional relevant toxicology information was found for the target substance.

3.1.2 Review of toxicological data

3.1.2.1 Acute and local toxicity

Oral toxicity

The acute oral toxicity of azamethiphos has been tested in rats at doses up to 2000 mg/kg body weight, and an oral median lethal dose (LD_{50}) of 500 mg/kg body weight (bw) was derived. The oral LD_{50} value of 500 mg/kg bw for female rats is within the criteria of LD50 >300 to ≤2000 mg/kg bwt for classification and is therefore classified for acute oral toxicity. Based on the LD_{50} value, an Acute Toxicity Estimate (ATE) of 500 mg/kg bw is proposed.

Dermal toxicity

The acute dermal toxicity of azamethiphos has been tested in rats at doses up to 2000 mg/kg body weight (OECD 402), and no mortality was observed. Therefore, the dermal LD_{50} was derived as >2000 mg/kg body weight. The LD50 of >2000 mg/kg bw for rats exposed to azamethiphos via the dermal exposure route is above the value for classification (2000 mg/kg), so it is not classified for dermal toxicity.

The skin irritation potential of azamethiphos has been tested in rabbits (OECD 404) and no significant skin irritation was observed. Azamethiphos did not cause either erythema or oedema (all scores were 0) in any of the animals tested. Therefore, the criteria for classification as a skin irritant are not met.

The skin sensitisation potential of azamethiphos has been tested in mice in the Local Lymph Nose Assay (LLNA) (OECD 429). Azamethiphos induced a positive response in an LLNA study, with Stimulation Index (SI) values of 14.1, 18.6 and 16.4 for concentrations of 10%, 25% and 50% azamethiphos, respectively. It therefore meets the criteria for classification as a skin sensitiser.

Inhalatory toxicity

The acute inhalation toxicity of azamethiphos has been tested in rats at concentrations up to 5.2 mg/L as a dust aerosol (OECD 403). The median lethal concentration (LC_{50}) was derived as between 0.5 and 1.0 mg/L for male and female rats. The inhalation LC50 value of 0.5 – 1.0 mg/l with a mass median aerodynamic diameter (MMAD) in the range of 2.3 - 2.9 µm, is within the numeric criteria of $0.5 < LC_{50} \leq 1$mg/l (dusts and mists) for classification for acute inhalation toxicity. Since no precise LC_{50} value is available, the default ATE value is proposed. In accordance with Annex I of the CLP Regulation, an ATE of 0.5 mg/l is appropriate for dusts and mists classified in category 3 for acute toxicity via the inhalation route.

Table 3.2 Azamethiphos classifications

Endpoint	Classification
Acute toxicity – oral route	Acute Tox 4; H302 – Harmful if swallowed ATE oral: 500 mg/kg bw
Skin sensitisation	Skin Sens 1; H317 – May cause an allergic skin reaction
Acute toxicity - inhalation route	Acute Tox 3; H331 – Toxic if inhaled ATE inhalation = 0.5mg/l

Conclusion: As azamethiphos is classified for acute oral and acute inhalatory toxicity, the dose-response assessment factor of the DNEL calculation has been adjusted to incorporate this local toxicity risk into the systemic DNEL.

3.1.2.2 Genotoxicity and carcinogenicity

Details of relevant studies are presented in Table 3.3. The outcome of any reliability assessments conducted on the studies listed is unknown and there was no opportunity to conduct *de novo* Klimisch assessments as the original data were not available.

Four *in vitro* genotoxicity studies were conducted with azamethiphos, and positive results were observed in all four assays. Azamethiphos showed mutagenic potential in bacteria in the absence of metabolic activation and was positive for mutagenic activity and clastogenicity in mammalian cells. Additionally, an *in vitro* comet assay was conducted and showed DNA damage in mouse lymphoma cells.

Three *in vivo* genotoxicity studies were conducted with azamethiphos, and negative results were observed in all three assays. Azamethiphos was negative for clastogenicity in an *in vivo* micronucleus assay and negative for DNA damage in both an unscheduled DNA synthesis assay and an *in vivo* comet assay. Based on the three robust *in vivo* negative results, showing that azamethiphos did not induce micronuclei or DNA damage in mammals, the substance is considered non mutagenic.

The CLH report for azamethiphos also concluded that azamethiphos should not be classified for mutagenicity (EC 2018).

The carcinogenic potential of azamethiphos has been investigated in five studies; a 24-month combined chronic/carcinogenicity study, two 2-year carcinogenicity studies in rats and two lifetime carcinogenicity studies in mice. In the combined chronic toxicity/carcinogenicity study in rats, an increased incidence of leiomyoma of the jejunum was observed in females treated with azamethiphos (1/50, 2/50 and 2/50 in the 0.05, 0.5 and 5 mg/kg/day groups, respectively). However, there was no clear dose-response observed and no statistically significant increase compared to the control group. Additionally, an increased incidence of endometrial adenocarcinoma was observed in females treated with 5 mg/kg/day azamethiphos. However, the increase was not statistically significant compared to the control group. Therefore, these findings were considered incidental. No treatment-related neoplastic findings were observed in any of the other studies conducted, which were carried out at higher doses. Therefore, based on the five available carcinogenicity studies for azamethiphos, there was no clear evidence of a consistent neoplastic effect.

The CLH report for azamethiphos also concluded that azamethiphos should not be classified for carcinogenicity (EC 2018). However, ECHA's response indicated that the substance should be classified as Carc. 2; H351. This was due to the occurrence of leiomyomas in the jejunum and endometrial adenocarcinomas in female rats, and the Risk Assessment Committee (RAC) concluded there is some (limited) evidence for carcinogenicity, supporting a Carc. 2; H351 classification (RAC 2019).

Conclusion: As azamethiphos is not classified for mutagenicity or carcinogenicity, no specific adjustments for this endpoint are required to the assessment factors in the systemic DNEL derivation.

Table 3.3 Azamethiphos - Summary of relevant genetic/carcinogenic toxicity data

Study type	Test substance	Experimental design	Results	Reference
In vitro bacterial reverse mutation assay (OECD 471)	Azamethiphos (96.2% pure)	Test item concentrations ranging from 50 to 500 µg/ml (-S9 mix) and 5 to 160 µg/ml (+S9 mix) tested for mutagenic activity, in *S. Typhimurium* strains (TA1535, TA1537, TA98, TA100) and *Escherichia coli* strains (WP2uvrA) with and without metabolic activation.	Positive. A dose-dependent increase in revertant colonies observed in TA100 strain without metabolic activation.	EC 2018; Verspeek-Rip (2008)
In vitro mammalian cell chromosome aberration test (OECD 473)	Azamethiphos (96.2% pure)	Test item concentrations up to 600 µg/ml tested in cultured peripheral human lymphocytes for the presence of chromatid-type and chromosome-type aberrations, with and without metabolic activation.	Positive. A dose-dependent increase in cells with structural chromosome aberrations and polyploid cells with and without metabolic activation.	EC 2018; Drs Buskens C.A.F. (2008b)
In vitro gene mutation assay in mammalian cells (OECD 476)	Azamethiphos (96.2% pure)	Test item concentrations up to 500 µg/ml tested for mutagenic activity in mouse lymphoma cells, with and without metabolic activity.	Positive. A dose-dependent increase in mutation frequency at the TK locus, with and without metabolic activation.	EC 2018; Verspeek-Rip (2008)
In vitro mammalian cell alkaline comet assay	Azamethiphos (99.68% pure)	Test item concentrations of 62.5, 125 and 250 µg/ml tested for DNA damage in mouse lymphoma cells.	Positive. A dose-dependent increase in percentage of DNA in tail without metabolic activation.	EC 2018; Simlar (2017)
In vivo mammalian bone marrow micronucleus test (OECD 474)	Azamethiphos	Groups of 30 male mice/dose were treated with doses of 30, 60 and 125 mg/kg via oral gavage on two consecutive days. Femoral bone marrow cells were then examined for polychromatic erythrocytes and occurrence of micronuclei was determined.	Negative. No increase in the mean micronuclei or polychromatic erythrocytes observed.	EC 2018; Confidential (2008)
In vivo unscheduled DNA synthesis test in rats (OECD 486)	Azamethiphos	Groups of 3 rats/group were treated with a single oral gavage at doses of 0, 425 and 850 mg/kg. Hepatocytes were then harvested 2 to 4 or 14 to 16 hours after dosing and examined for unscheduled DNA synthesis.	Negative. No increase in hepatocyte DNA repair observed.	EC 2018; Confidential (2008)
In vivo rat stomach and duodenum comet assay (OECD 489)	Azamethiphos (99.68% pure)	Groups of 5 male rats/group were treated with a single oral gavage at doses of 0, 50, 100 and 200 mg/kg. Samples then collected from the stomach and duodenum and analysed for DNA damage.	Negative. No increase in percentage of DNA in tail observed.	EC 2018; Confidential (2017)
Combined chronic toxicity / carcinogenicity	Azamethiphos	Groups of 50 rats/sex/group were treated with doses of 0, 0.05, 0.5 and 5 mg/kg/day via the diet for periods of up to 12 months (chronic toxicity) and 2 years (carcinogenicity).	No non-neoplastic effects observed. Increased incidence of leiomyoma of the jejunum and endometrial	EC 2018; Confidential

Study type	Test substance	Experimental design	Results	Reference
study in rats (OECD 453)			adenocarcinoma observed in females. No clear NOAEL identified	(2011); Klimisch 1
Two year carcinogenicity study in rats	Azamethiphos (95.6% pure)	Groups of 60 rats/sex/group were treated with doses of 0, 15, 60 and 327 ppm (0, 0.8, 3 and 16 mg/kg/day) via the diet for periods of up to 2 years.	Effects on body weights, kidney lesions and cholinesterase activity observed. No neoplastic effects observed. NOAEL given as 15 ppm (0.8 mg/kg/day)	EC 2018; Confidential (1982); Klimisch 2
Two year carcinogenicity study in rats (OECD 409)	Azamethiphos	Groups of 50 rats/sex/group were treated with doses of 0, 20, 200 and 1500 ppm (0, 0.8/1.1, 8.2/11.2 and 62.2/88.7 mg/kg/day in males/females, respectively) via the diet for periods of up to 2 years.	Effects on body weights, kidney weights, liver lesions and cholinesterase activity observed. No neoplastic effects observed. NOAEL given as 200 ppm (8/11.2 mg/kg/day in males/females).	EC 2018; Confidential (1989); Klimisch 1
Lifetime carcinogenicity study in mice (OECD 451)	Azamethiphos	Groups of 51 mice/sex/group were treated with doses of 0, 50, 500, 1500 and 4000 ppm (0/0, 6.2/7.7, 60.2/76.2, 183.4/219.7 and 491.4/582.9 mg/kg/day in males/females, respectively) via the diet for the lifetime.	Effects on survival, body weights and small intestine lesions observed. No neoplastic effects observed. NOAEL was not identified.	EC 2018; Confidential (1989); Klimisch 1
Lifetime carcinogenicity study in mice (OECD 451)	Azamethiphos	Groups of 60 mice/sex/group were treated with doses of 0, 11, 97 and 396 ppm (0, 2, 14 and 57 mg/kg/day) via the diet for the lifetime.	Effects on survival. No neoplastic effects observed. NOAEL was not identified.	EC 2018; Confidential (1982); Klimisch 2

3.1.2.3 Repeat-dose toxicity

Details of relevant studies are presented in Table 3.4. The outcome of any reliability assessments conducted on the studies listed is unknown and there was no opportunity to conduct *de novo* Klimisch assessments as the original data were not available.

Subchronic and chronic toxicity studies

From a report conducted by the European Medicines Agency (EMA 1999), four repeat dose toxicity studies were reported, three conducted in dogs (90 day, 90 day and 52 week) and one conducted in rats (90 day). In one of the 90-day toxicity studies conducted in dogs, groups of 4 to 5 male and female Beagles were treated with azamethiphos via the diet at concentrations of 0, 30, 300 and 3000 mg azamethiphos/kg feed for a period of 90 days. The highest dose level was reduced to 1000 mg/kg feed on day 36 due to severe bodyweight loss. Effects were observed as emesis and decreased plasma and erythrocyte cholinesterase activity at all dose levels, therefore a NOEL could not be established.

A second 90-day toxicity study in dogs at concentrations of 0 and 10 mg/kg feed (0.26/0.33 mg/kg/day azamethiphos in males/females, respectively) showed no treatment related effects. However, it was considered that the single dose level used in this study could not be formally established as a NOEL.

In a chronic toxicity study in dogs, groups of 4 male and female Beagles were treated with azamethiphos via the diet at concentrations of 0, 10, 100 or 1000 mg/kg feed (0/0, 0.26/0.24, 2.72/2.86 and 31.5/28.43 in males/females, respectively) for a period of 52 weeks. At 1000 mg/kg feed, decreased brain, plasma and erythrocyte cholinesterase activities were observed and at 100 mg/kg feed, decreased plasma and erythrocyte cholinesterase activities were observed. However, the effects at 100 mg/kg feed were considered not to be biologically significant, therefore the NOAEL was estimated as 100 mg/kg feed (2.72 and 2.86 mg/kg/day in males and females, respectively).

In a 90-day toxicity study conducted in rats, groups of 25 male and female Sprague-Dawley rats were treated with azamethiphos via the diet at concentrations of 0, 30, 300 or 3000 mg/kg feed (0, 2, 20, 241 mg/kg/day, respectively) via the diet for a period of 13 weeks. Effects were observed on decreased plasma and erythrocyte cholinesterase activity at all dose levels, therefore a NOEL could not be established.

The ECHA CLH report (EC 2018) presents four repeat dose toxicity studies, three conducted in rats (28-day, 90-day and combined chronic toxicity/ carcinogenicity) and one conducted in dogs (90-day).

In the 28-day study in rats, groups of 3 rats/sex/group were treated with doses of 0, 0.5, 5 and 50 mg/kg/day via oral gavage for a period of 28 days. The main adverse effect was cholinesterase inhibition in females at doses of 5 mg/kg/day and males and females at 50 mg/kg/day. Clinical signs such as lethargy, calm behaviour, tremors, flat/hunched posture, uncoordinated movement, piloerection and/or salivation were also observed, however, they were not treatment related. Therefore, the NOAEL can be estimated to be 0.5 mg/kg/day.

In the 90-day toxicity study in rats, groups of 15 rats/sex/group were treated with doses of 0, 0.05, 0.5 and 5 mg/kg/day via oral gavage for a period of 90 days. The main effect observed was on cholinesterase inhibition which was >20% in both sexes at the top dose. It was also found to be slightly above the level considered adverse in mid-dose group females at 8 weeks (22%), however this finding was not present at 13 weeks (4.9%). Therefore, the NOAEL can be estimated to be 0.05 mg/kg/day.

In the combined chronic toxicity / carcinogenicity study in rats, groups of 50 rats/sex/group were treated with doses of 0, 0.05, 0.5 and 5 mg/kg/day via the diet for periods of up to 12 months (chronic toxicity) and 2 years (carcinogenicity). The key effect observed was cholinesterase inhibition in males and females treated with 5 mg/kg/day. Therefore, the NOAEL can be estimated to be 0.5 mg/kg/day.

In the 90-day toxicity study in dogs, groups of 4 dogs/sex/group were treated with doses of 0, 0.2, 2 and 20 mg/kg/day via oral gavage for a period of 90 days. At 20 mg/kg/day, cholinesterase inhibition and associated transient clinical signs were observed. At 2 mg/kg/day, cholinesterase inhibition was observed in males. Therefore, the NOAEL can be estimated to be 0.2 mg/kg/day.

Developmental and reproductive toxicity studies

The European Medicines Agency (EMA 1999) report four developmental studies and two two-generation reproduction studies in rats, rabbits and chinchillas.

In the first developmental toxicity study conducted in rats, one group of 22 – 29 female rats were treated with doses of 0, 25, 75 and 150 mg/kg/day via oral gavage on gestation days 6 to 15. Maternal toxicity was observed in this study at 150 mg/kg/day, as was decreased food consumption. There was no evidence of teratogenicity, however, an increased incidence of delayed ossification was observed at 75 mg/kg/day. Therefore, the NOAEL was given as 75 mg/kg/day.

In the second rat developmental toxicity study, groups of 26 – 29 female rats were treated with doses of 0, 1, 75 and 200 mg/kg/day via oral gavage on gestation days 6 to 15. Maternal toxicity was observed at 200 mg/kg/day as increased clinical signs (salivation, lethargy and diarrhoea) and decreased body weight. There was no evidence of teratogenicity or foetal toxicity, therefore, the NOAEL was given as 75 mg/kg/day.

In the developmental toxicity study in rabbits, groups of female New Zealand White rabbits were treated with doses of 0, 2, 12 and 36 mg/kg/day via oral gavage on gestation days 7 to 19. The dose level of 36 mg/kg/day was reduced to 18 mg/kg/day due to the deaths of 4 dams. Maternal toxicity was observed at 12 and 18 mg/kg/day as decreased body weights. There was no evidence of teratogenicity or foetal toxicity, therefore, the NOAEL was given as 2 mg/kg/day.

Additionally, a developmental toxicity study was conducted in the chinchilla in which groups of female chinchillas treated with doses of 0, 2.5, 7.5 and 15 mg/kg/day via oral gavage on

gestation days 6 to 18. Foetal toxicity was observed in the study as decreased foetal weights and delayed ossification at 15 mg/kg/day. The NOAEL was given as 7.5 mg/kg/day.

Two two-generation reproduction toxicity studies were conducted in rats. In the first study, groups of rats were treated with doses of 0, 40, 200 and 1000 mg/kg feed (0/0, 2.7/3.0, 13.3/14.8 and 65.2/71.3 mg/kg/day in males/females, respectively) via the diet for two generations. No significant effects were observed on mating or fertility. Parental toxicity was observed at 200 and 1000 mg/kg feed as decreased body weights and cholinesterase inhibition, with corresponding decreases in pup weights. Therefore, the parental and offspring NOAELs were given as 40 mg/kg feed (2.7/3.0 mg/kg/day for males/ females, respectively), and the reproductive NOAEL was given as 1000 mg/kg feed (65/71 mg/kg/day in males/females, respectively).

In the second study, groups of rats were treated with dose levels of 0, 2, 40, 200 and 1000 mg/kg feed (0, 0.1, 2 – 3, 10 and 50 mg/kg/day, respectively) via the diet for two generations. No significant effects were observed on mating or fertility in either study. Parental toxicity was observed at 200 and 1000 mg/kg feed as decreased body weights, with corresponding decreases in pup weights. Therefore, the parental and offspring NOAELs were given as 40 mg/kg feed (2-3 mg/kg/day), and the reproductive NOAEL was given as 1000 mg/kg feed (50 mg/kg/day).

Two developmental toxicity studies are reported in the ECHA CLH report (EC 2018), one in rats and one in rabbits, as well as one two-generation reproduction toxicity study in rats.

In the developmental toxicity study in rats, groups of 24 female Sprague Dawley rats were treated with dose levels of 0, 0.1, 1 and 10 mg/kg/day via oral gavage on gestation days 6 to 20. There were no treatment related effects on mortality, clinical signs, body weight, food consumption or embryo-foetal development at any dose. Cholinesterase inhibition was observed in maternal rats treated with 10 mg/kg/day. Therefore, the NOAEL can be estimated as 1 mg/kg/day.

In the developmental toxicity study in rabbits, groups of 26 female New Zealand White rabbits were treated with dose levels of 0, 0.05, 0.5 and 5 mg/kg/day via oral gavage on gestation days 7 to 29. There were no treatment related effects on mortality, clinical signs, body weight, food consumption or embryo-foetal development at any dose. Cholinesterase inhibition was observed in maternal rabbits treated with 5 mg/kg/day. Therefore, the NOAEL can be estimated as 0.5 mg/kg/day.

In the two-generation reproduction toxicity study in rats, groups of 24 Sprague Dawley rats were treated with dose levels of 0, 1, 10 and 100 mg/kg/day on study days 1 – 9, 0, 0.01, 0.1 and 1 mg/kg/day on study days 10 – 16 and 0, 0.05, 0.5 and 5 mg/kg/day on study day 17 onwards, via the diet for two generations. Effects on parental generations were observed as cholinesterase inhibition, decreased body weights and clinical signs at doses of 0.5 mg/kg/day and above. There were no treatment related effects on reproductive or developmental parameters. Therefore, the parental NOAEL was estimated as 0.05 mg/kg/day and the reproductive NOAEL was estimated as 5 mg/kg/day.

Conclusion: The NOAEL/NOEL selected as the PoD was 0.05 mg/kg/day taken from the 90-day oral gavage repeat-dose neurotoxicity study in rats (EC 2018; Confidential 2009).

Table 3.4 Azamethiphos - Summary of relevant repeat-dose toxicity data

Study type	Test substance	Experimental design	Results	Reference
90-day repeat dose toxicity study in dogs	Azamethiphos	Groups of 4 to 5 male and female Beagle dogs were treated with doses of 0, 30, 300 and 3000 mg/kg feed (0/0, 1.1/1.2, 11.0/12.8, ND/ND mg/kg/day in males/females, respectively) via the diet for a period of 13 weeks. The 3000 ppm group was reduced to 1000 ppm on day 36.	Effects observed on decreased body weights, emesis and decreased plasma and erythrocyte cholinesterase activities. A NOEL could not be established as effects were observed at all doses, however the LOEL was given as 1.1/1.2 mg/kg/day.	EMA 1999
90-day repeat dose toxicity study in dogs	Azamethiphos	Groups of 4 dogs/sex/group were treated with doses of 0 or 10 mg/kg feed (0/0 and 0.26/0.33 mg/kg/day in males/females, respectively) via the diet for a period of 13 weeks.	No adverse effects observed. NOEL not determined due to only one dose level.	EMA 1999
90-day repeat dose toxicity study in rats	Azamethiphos	Groups of 25 rats/sex/group were treated with doses of 0, 30, 300 or 3000 mg/kg feed (0, 2, 20, 241 mg/kg/day, respectively) via the diet for a period of 13 weeks.	Effects observed on plasma and erythrocyte cholinesterase activities. A NOEL could not be established as effects were observed at all doses, however the LOEL was given as 2 mg/kg/day.	EMA 1999
28-day repeat dose toxicity study in rats (OECD 407)	Azamethiphos (96.2% pure)	Groups of 3 rats/sex/group were treated with doses of 0, 0.5, 5 and 50 mg/kg/day via oral gavage for a period of 28 days.	Effects observed on clinical signs and cholinesterase activity. NOAEL estimated to be 0.5 mg/kg/day.	EC 2018; Confidential (2009)
90-day repeat dose / neurotoxicity study in rats (OECD 408 / 424)	Azamethiphos (96.2% pure)	Groups of 15 rats/sex/group were treated with doses of 0, 0.05, 0.5 and 5 mg/kg/day via oral gavage for a period of 90 days.	Effects observed on cholinesterase activity and clinical signs. NOAEL estimated to be 0.05 mg/kg/day.	EC 2018; Confidential (2009)
90-day repeat dose toxicity study in dogs (OECD 409)	Azamethiphos (96.2% pure)	Groups of 4 dogs/sex/group were treated with doses of 0, 0.2, 2 and 20 mg/kg/day via oral gavage for a period of 90 days.	Effects observed on clinical signs, liver weight and cholinesterase activity. NOAEL estimated to be 0.2 mg/kg/day.	EC 2018; Confidential (2011)
52-week chronic toxicity study in dogs	Azamethiphos	Groups of 4 dogs/sex/group were treated with doses of 0, 10, 100 or 1000 mg/kg feed (0/0, 0.26/0.24, 2.72/2.86	Effects observed on decreased brain, plasma and erythrocyte cholinesterase activities at highest	EMA 1999

Study type	Test substance	Experimental design	Results	Reference
		and 31.5/28.43 in males/females, respectively) via the diet for a period of 52 weeks.	dose. NOAEL given as 2.72/2.86 mg/kg/day in males/females, respectively.	
Combined chronic toxicity / carcinogenicity study in rats (OECD 453)	Azamethiphos	Groups of 50 rats/sex/group were treated with doses of 0, 0.05, 0.5 and 5 mg/kg/day via the diet for periods of up to 12 months (chronic toxicity) and 2 years (carcinogenicity).	Effects observed on decreased cholinesterase activity and tumour incidence. NOAEL estimated as 0.5 mg/kg/day.	EC 2018; Confidential (2011); Klimisch 1
Developmental toxicity study in rats	Azamethiphos	Groups of 22 – 29 female rats treated with doses of 0, 25, 75 and 150 mg/kg/day via oral gavage on gestation days 6 to 15.	Effects on maternal toxicity (decreased food consumption) and foetal delayed ossification. NOAEL given as 75 mg/kg/day.	EMA 1999
Developmental toxicity study in rats	Azamethiphos	Groups of 26 – 29 female rats treated with doses of 0, 1, 75 and 200 mg/kg/day via oral gavage on gestation days 6 to 15.	Effects on maternal toxicity (clinical signs and decreased body weight). No effects on foetal toxicity. NOAEL given as 75 mg/kg/day.	EMA 1999
Developmental toxicity study in rabbits	Azamethiphos	Groups of female New Zealand White rabbits treated with doses of 0, 2, 12 and 36 mg/kg/day via oral gavage on gestation days 7 to 19. Dose level of 36 mg/kg/day reduced to 18 mg/kg/day.	Effects on maternal toxicity (decreased survival and body weight). No effects on foetal toxicity. NOAEL given as 2 mg/kg/day.	EMA 1999
Developmental toxicity study in chinchillas	Azamethiphos	Groups of female chinchillas treated with doses of 0, 2.5, 7.5 and 15 mg/kg/day via oral gavage on gestation days 6 to 18.	Effects on foetotoxicity (decreased foetal weights and delayed ossification). NOAEL given as 7.5 mg/kg/day.	EMA 1999
Developmental toxicity study in rats (OECD 414)	Azamethiphos (96.2% pure)	Groups of 24 female Sprague Dawley rats treated with doses of 0, 0.1, 1 and 10 mg/kg/day via oral gavage on gestation days 6 to 20.	Effects on maternal toxicity (cholinesterase inhibition). No effects on foetal toxicity. NOAEL estimated as 1 mg/kg/day.	EC 2018; Confidential (2009)
Developmental toxicity study in rabbits (OECD 414)	Azamethiphos (96.2% pure)	Groups of 26 female New Zealand White rabbits treated with doses of 0, 0.05, 0.5 and 5 mg/kg/day via oral gavage on gestation days 7 to 29.	Effects on maternal toxicity (cholinesterase inhibition). No effects on foetal toxicity. NOAEL estimated as 0.5 mg/kg/day.	EC 2018; Confidential (2009)
Two-generation reproduction	Azamethiphos	Groups of rats treated with doses of 0, 40, 200 and 1000 mg/kg feed (0/0, 2.7/3.0, 13.3/14.8 and 65.2/71.3	Effects on parental toxicity (decreased bodyweight) and offspring toxicity (decreased	EMA 1999

Study type	Test substance	Experimental design	Results	Reference
toxicity study in rats		mg/kg/day in males/females, respectively) via the diet for two generations.	bodyweight). No effects on reproductive toxicity. Parental and offspring NOAEL given as 2.7/3.0 mg/kg/day in males/females, respectively. Reproductive NOAEL given as 65/71 mg/kg/day in males/females, respectively.	
Two-generation reproduction toxicity study in rats	Azamethiphos	Groups of rats treated with doses of 0, 2, 40, 200 and 1000 mg/kg feed (0, 0.1, 2 – 3, 10 and 50 mg/kg/day, respectively) via the diet for two generations.	Effects on parental toxicity (decreased bodyweight and cholinesterase inhibition) and offspring toxicity (decreased bodyweight). No effects on reproductive toxicity. Parental NOAEL given as 2 – 3 mg/kg/day. Offspring NOAEL given as 10 mg/kg/day. Reproductive NOAEL given as 50 mg/kg/day.	EMA 1999
Two-generation reproduction toxicity study in rats (OECD 416)	Azamethiphos	Groups of 24 Sprague Dawley rats treated with doses of 0, 1, 10 and 100 mg/kg/day on days 1 – 9, 0, 0.01, 0.1 and 1 mg/kg/day on days 10 – 16 and 0, 0.05, 0.5 and 5 mg/kg/day on days 17 onwards, via the diet for two generations.	Effects on parental toxicity (cholinesterase inhibition, clinical signs, decreased body weight). No effects on reproductive toxicity. Parental NOAEL estimated as 0.05 mg/kg/day. Reproductive NOAEL estimated as 5 mg/kg/day.	EC 2018; Confidential (2009)

3.1.3 DNEL Calculation

All the relevant toxicity studies with azamethiphos were conducted by the oral route of administration, either via dietary inclusion with 24 hour/day *ad libitum* availability or by daily oral gavage administration. Consequently, the PoD for all DNEL calculations will be based on an oral NOAEL/NOEL from an appropriate study.

The NOAEL/NOEL selected as the PoD was 0.05 mg/kg/day taken from the 90-day oral gavage repeat-dose neurotoxicity study in rats (EC 2018; Confidential 2009). This study was selected based on the following reasoning:

- The oral gavage route of exposure was preferred over dietary administration of the test substance, as an accurate peak dose exposure was achieved for each individual rat in such studies.
- The 90-day repeat-dose test system is accepted as a good level of predictiveness of the potential exposure associated with the open-water swimming scenario.
- A dose level of 0.05 mg/kg/day represents the most conservative PoD from other studies of this type (e.g. 28-day toxicity study in rats by daily oral gavage).
- There were no carcinogenicity effects at dietary inclusion levels equivalent to up to at least 88.7 mg/kg/day, and there were negative results for mutagenicity/clastogenicity in *in vitro* assays.
- In the chosen study, the toxicological effect on cholinesterase inhibition is observed at doses above the derived NOAEL. Cholinesterase inhibition is a very variable parameter between mammalian species and, using this parameter as a determining factor of adverse effect does have a considerable influence on the choice of PoD. However, by using the 90-day rat neurotoxicity study, the resulting PoD shows no adverse effects on brain cholinesterase and no associated clinical effects.
- The PoD does not specifically take into consideration the period of pregnancy or other reproductive toxicological endpoints. However, the most conservative NOAEL in developmental toxicity studies in which cholinesterase inhibition was investigated is 1 mg/kg/day.

Under other regulations, alternative health-based exposure limits have been derived. These would not be appropriate for use as DNELs as they represent lifetime exposure. The European Medicines Agency (EMA) report an oral long-term Accepted Daily Intake (ADI) of 0.025 mg/kg bw for azamethiphos. This was based on the NOAEL of 2.5 mg/kg/day from the 52-week toxicity study in dogs (EMA 1999). Alternatively, the FDA report an ADI of 0.001 mg/kg bw/day, based on the NOAEL of 0.1 mg/kg/day from the 2-generation rat reproductive toxicity study via dietary administration.

The general formula for the calculation of an Endpoint-specific DNEL is as follows:

- $DNEL_{Endpoint\ specific} = NOAEL_{corrected}/AF_1 \times AF_2 \times AF_3 \times AF_4 \times AF_5$

Where;
$NOAEL_{corrected}$ is the PoD corrected for the appropriate route of administration and any known differences in absorption kinetics.
AF are the various Assessment Factors (see below).

Oral

Selection of relevant dose-descriptor: PoD 0.05 mg/kg/day (as indicated above)

Modification of relevant dose-descriptor: Dose descriptor modification is not considered appropriate in this case since the human exposure route is the same as that in experimental animals and the experimental exposure conditions are considered to be similar to the swallowing of contaminated water by an open-water swimmer.

Since the start and end route of exposure are the same and no specific absorption data is available, it will be assumed that there is 100% absorption as the worst-case-scenario default.

Application of assessment factors:

- Interspecies differences - apply a factor for allometric scaling (4 for rat) for $oral_{rat}$ to $oral_{human}$ exposure
- Intraspecies differences – use Worker default of 5 as this will replicate short term exposure in a light-work scenario (open-water swimmer).
- Differences in duration of exposure - PoD taken from a subacute toxicity study (90-days duration) and this daily exposure is considered representative of the frequency of exposure in open water swimming. Therefore, AF is 1.
- Issues related to dose response – PoD based on NOAEL since dose related inhibition of peripheral blood cholinesterase levels were reported. In addition, as this dose level is not associated with changes in brain cholinesterase activity or associated clinical observations. Azamethiphos is classified as Acute Tox 4; H302 – Harmful if swallowed and Acute Tox 3; H331 – Toxic if inhaled, therefore, an AF of 2 is used to account for this local toxicity.
- Quality of whole database – several oral toxicity studies in rodents are available for evaluation including the preferred route of administration of oral gavage. All of the studies listed in Tables 3.3 and 3.4 are of good quality and reliability, as indicated by the Klimisch scores (reported where available). Consequently, the standard default assessment factor of 1 should be applied.

Therefore;

- $DNEL_{Oral} = NOAEL_{corrected}/AF1 \times AF2 \times AF3 \times AF4 \times AF5$

  $= 0.05/4 \times 5 \times 1 \times 2 \times 1$

= **0.00125 mg/kg/day**

Dermal

Selection of relevant dose-descriptor: PoD 0.05 mg/kg/day (as indicated above)

Modification of relevant dose-descriptor: Convert rat oral NOAEL dose-descriptor into dermal rat NOAEL by correcting for differences in absorption between the two routes. As there is no specific absorption data available by these routes of administration, it will be assumed that there is 100% absorption as the worst-case scenario and no modification of the dose-descriptor is required.

Application of assessment factors:

- Interspecies differences – apply a factor for allometric scaling (4 for rat) for $oral_{rat}$ to $dermal_{human}$ exposure.
- Intraspecies differences - use Worker default of 5 as this will replicate short term exposure in a light-work scenario (open water swimmer).
- Differences in duration of exposure – PoD taken from a subacute toxicity study (90-days duration) and this daily exposure is considered representative of the frequency of exposure in open water swimming. Therefore, AF is 1.
- Issues related to dose response - PoD based on NOAEL since dose related inhibition of peripheral blood cholinesterase levels were reported. In addition, as this dose level is not associated with changes in brain cholinesterase activity or associated clinical observations, and there are no dermal local toxicity classifications, the AF is 1.
- Quality of whole database – several oral toxicity studies in rodents are available for evaluation including the preferred route of administration of oral gavage. All of the studies listed in Tables 3.3 and 3.4 are of good quality and reliability, as indicated by the Klimisch scores (reported where available). Consequently, the standard default assessment factor of 1 should be applied.

Therefore;

- $DNEL_{Dermal}$ = $NOAEL_{corrected}$/AF1 x AF2 x AF3 x AF4 x AF5

  = 0.05/4 x 5 x 1 x 1 x 1

  = **0.0025 mg/kg/day**

3.2 Deltamethrin

3.2.1 Hazard assessment

3.2.1.1 Regulatory data

Deltamethrin is not a registered chemical under REACH, however, it is a registered plant protection product therefore a Draft Renewal Assessment Report for Deltamethrin was available through EFSA (EC 2017). Sufficient toxicological data was available in this report in order to complete this assessment.

3.2.1.2 Literature search

The search was conducted using the substance name and identifiers, with an appropriate search string and the results of the search are summarised in Table 3.5. Results were not date limited.

Table 3.5 Deltamethrin - Literature search results

Search	Number of hits		Combined number of hits after duplicates removed
	Web of Science	PubMed	
(Deltamethrin) AND (Toxicology OR toxicity OR Chronic OR Subchronic OR Sublethal OR NOAEL OR LOAEL OR Repro* OR Carcinogen* OR Mutagen* OR Genotox* OR Oral OR Dermal OR Inhal* OR Pharmacokinetic OR Toxicokinetic) AND (Rat OR Mouse OR Human OR Dog OR Rabbit OR In vitro)	603	650	966

Where references were identified, the resulting references from the searches were downloaded into an Excel spreadsheet as a record. The titles and abstracts of the articles were then screened for potentially relevant articles. A total of 12 articles were obtained and assessed for relevance, however, no additional relevant toxicology information was identified.

3.2.2 Review of toxicological data

3.2.2.1 Acute and local toxicity

Oral toxicity

The acute oral toxicity of deltamethrin has been tested in a number of studies. The acute oral toxicity in rats was high when peanut oil was used as the vehicle (LD50: 52 and 31 mg/kg bw for males and females, respectively). A number of other studies using oil-based vehicles produced acute oral LD50 values in the range 67 - 200 mg/kg bw. The acute oral LD50 in weanling rats was 50 mg/kg bw, similar to the values for adult animals. These values are consistent with the current classification of deltamethrin for acute oral toxicity.

Dermal toxicity

The acute dermal toxicity of deltamethrin has been tested in three studies. In all studies, there were no deaths at 2000 mg/kg bw or above. Therefore, deltamethrin is not classified for acute dermal toxicity.

The skin irritation potential of deltamethrin has been tested in rabbits and no significant skin irritation was observed in four tests. Deltamethrin was not a skin sensitizer in two guinea pig Maximisation Tests or in two Buehler tests in guinea pigs. Therefore, deltamethrin is not classified for skin irritation nor for skin sensitisation.

Inhalatory toxicity

The acute inhalation toxicity of deltamethrin has been tested in rats. The studies reported LC_{50} values ranging from 0.6 to 3.3 mg/L, and these are consistent with classification of deltamethrin for acute inhalation toxicity.

Table 3.6 Deltamethrin classifications

Endpoint	Classification
Acute toxicity – oral route	Acute Tox 3; H301 – Harmful if swallowed
Acute toxicity - inhalation route	Acute Tox 3; H331 – Toxic if inhaled

Conclusion: As deltamethrin is classified for acute oral and acute inhalatory toxicity, the dose-response assessment factor of the DNEL calculation has been adjusted to incorporate this local toxicity risk into the systemic DNEL.

3.2.2.2 Genotoxicity and carcinogenicity

Details of relevant studies are presented in Table 3.7. The outcome of any reliability assessments conducted on the studies listed is unknown and there was no opportunity to conduct *de novo* Klimisch assessments as the original data was not available.

Deltamethrin showed no evidence of genotoxicity from the available assays. A total of six *in vitro* assays were conducted with deltamethrin, and three *in vivo* assays. Deltamethrin was negative for mutagenic activity in bacteria in three *in vitro* bacterial reverse mutation assays, negative for mutagenic activity in mammalian cells and negative for chromosome aberration and micronuclei induction in mammalian cells. Three *in vivo* genotoxicity studies were conducted with deltamethrin, and negative results were observed in all three assays. Deltamethrin was negative for clastogenicity in two *in vivo* micronucleus tests and one *in vivo* chromosome aberration assay. Therefore, based on the *in vitro* and *in vivo* results, deltamethrin is considered non-mutagenic.

A draft Renewal Assessment Report (EC 2017) on deltamethrin also concluded that deltamethrin should not be classified for mutagenicity and indicated that this is consistent with the harmonised classification for deltamethrin.

The carcinogenic potential of deltamethrin has been investigated in four two-year feeding carcinogenicity studies; two in rats and two in mice. In the first carcinogenicity study in mice, increased clinical signs (dyspnoea and emaciation), decreased body weights and increased skin lesions (ulceration and cellulitis) were observed in mice and no carcinogenic effects were observed. In the second carcinogenicity study in mice, no adverse effects or carcinogenic effects were observed. In the first carcinogenicity study in rats, increased clinical signs (neurological effects), decreased body weights, decreased food consumption, and alterations in haematological and biochemical parameters were observed, but no carcinogenic effects were observed. In the second carcinogenicity study in rats, no adverse effects or carcinogenic effects were observed. Therefore, based on the four available carcinogenicity studies for azamethiphos, there was no evidence of a carcinogenic or neoplastic effect.

The Draft Renewal Assessment Report conducted for deltamethrin (EC 2017) also concluded that deltamethrin should not be classified for carcinogenicity.

Conclusion: As deltamethrin is not classified for mutagenicity or carcinogenicity, no specific adjustments for this endpoint are required to the assessment factors in the systemic DNEL derivation.

Table 3.7 Deltamethrin - Summary of relevant genetic/carcinogenic toxicity data

Study type	Test substance	Experimental design	Results	Reference
In vitro bacterial reverse mutation assay	Deltamethrin	Test item concentrations ranging from 2 to 5000 µg/plate tested for mutagenic activity, in *S. Typhimurium* strains (TA1535, TA1537, TA98, TA100), with and without metabolic activation.	Negative.	EC 2017; Peyre et al. (1980)
In vitro bacterial reverse mutation assay	Deltamethrin	Test item concentrations ranging from 156 to 5000 µg/plate tested for mutagenic activity, in *S. Typhimurium* strains (TA1537, TA98, TA100, TA102) and *E. coli* (WP2), with and without metabolic activation.	Negative.	EC 2017; Watanabe (2005)
In vitro bacterial reverse mutation assay	Deltamethrin	Test item concentrations ranging from 313 to 5000 µg/plate tested for mutagenic activity, in *S. Typhimurium* strains (TA1537, TA1535, TA98, TA100, TA102), with and without metabolic activation.	Negative.	EC 2017; Patel (2009)
In vitro gene mutation assay in mammalian cells	Deltamethrin	Test item concentrations ranging from 62 to 2000 µg/ml tested for mutagenic activity in V79 Chinese hamster cells, with and without metabolic activity.	Negative.	EC 2017; Wollny (2016)
In vitro micronucleus test in mammalian cells	Deltamethrin	Test item concentrations of 5.4 to 16.6 µg/ml (4h; -S9), 11.7 to 26.3 µg/ml (20h; -S9) and 16.6 to 50.8 µg/ml (4 h; +S9) tested for clastogenic activity in human lymphocytes for periods of 4 and 20 hours, with and without metabolic activation.	Negative.	EC 2017; Nauman (2016)
In vitro chromosome aberration assay	Deltamethrin	Test item concentrations of 0, 9.5, 19, 38, 75 and 150 µg/ml tested for clastogenic activity in Chinese Hamster Ovary cells, with and without metabolic activation.	Negative.	EC 2017; Putman and Morris (1989)
In vivo mammalian bone marrow micronucleus test	Deltamethrin	Groups of male mice were treated with a dose of 16 mg/kg via oral gavage. Bone marrow cells were then examined for polychromatic erythrocytes and occurrence of micronuclei was determined.	Negative.	EC 2017; Confidential (1983)
In vivo mammalian bone marrow micronucleus test (OECD 474)	Deltamethrin	Groups of male mice were treated with doses of 9.25, 18.5 and 37 mg/kg via oral gavage for two days. Bone marrow cells were then examined for polychromatic erythrocytes and occurrence of micronuclei was determined.	Negative.	EC 2017; Confidential (2009)

Study type	Test substance	Experimental design	Results	Reference
In vivo mammalian bone marrow chromosomal aberration test (OECD 475)	Deltamethrin	Groups of male mice were treated with doses of 5, 10 and 20 mg/kg via oral gavage for two days. Bone marrow cells were then examined for chromosomal aberrations.	Negative.	EC 2017; Confidential (2009)
Two-year carcinogenicity study in mice	Deltamethrin	Groups of male and female Swiss mice treated with concentrations of 0, 10, 100, 1000 and 2000 ppm orally via the diet for periods of up to 2 years.	Effects observed on clinical signs, body weight gain and skin lesions. No carcinogenic effects observed. NOAEL given as 100 ppm in females (16 mg/kg/day) and 1000 ppm in males (189 mg/kg/day).	EC 2017; Confidential (1995)
Two-year carcinogenicity study in mice	Deltamethrin	Groups of male and female Charles River CD-1 mice treated with concentrations of 0, 1, 5, 25 and 100 ppm orally via the diet for periods of up to 2 years.	No adverse effects observed. No carcinogenic effects observed. NOAEL given as 100 ppm in males (12 mg/kg/day) and females (15 mg/kg/day).	EC 2017; Confidential (1980)
Two-year carcinogenicity study in rats	Deltamethrin	Groups of male and female Charles River Crl:CD(SD)BR rats treated with concentrations of 0, 25, 125, 500 and 800 ppm orally via the diet for periods of up to 2 years.	Effects observed on clinical signs, body weight gain, food consumption, haematological and biochemical parameters. No carcinogenic effects observed. NOAEL given as 25 ppm in males (1 mg/kg/day) and 500 ppm in females (30 mg/kg/day).	EC 2017; Confidential (1995)
Two-year carcinogenicity study in rats	Deltamethrin	Groups of male and female Charles River CD rats treated with concentrations of 0, 2, 20 and 50 ppm orally via the diet for periods of up to 2 years.	No adverse effects observed. No carcinogenic effects observed. NOAEL given as 50 ppm in males (2.1 mg/kg/day) and females (2.8 mg/kg/day).	EC 2017; Confidential (1980)

3.2.2.3 Repeat-dose toxicity

Details of relevant studies are presented in Table 3.8. The outcome of any reliability assessments conducted on the studies listed is unknown and there was no opportunity to conduct *de novo* Klimisch assessments as the original data was not available.

Subchronic and chronic toxicity studies

From the Draft Renewal Assessment Report conducted for deltamethrin (EC 2017), seven repeat dose toxicity studies were reported, one conducted in mice (12-week), two conducted in rats (13-week and 13-week) and four conducted in dogs (13-week, 13-week, 52-week and 2-year).

In the 12-week toxicity study conducted in mice, groups of mice were treated with deltamethrin at concentrations of 0, 30, 300, 3000 and 6000 ppm (equivalent to 0/0, 6/8, 62/77, 603/739 and 1318/1391 mg/kg/day in males/females, respectively) orally via the diet. Effects were observed as increased mortality, increased incidence of adverse clinical signs (poor condition and neurological effects), decreased body weights and decreased food consumption. The NOAEL was given as 300 ppm (62/77 mg/kg/day in males/females, respectively).

Two 13-week toxicity studies were conducted in rats, one conducted via dietary administration and one via oral gavage. In the first 13-week week toxicity study conducted in rats, groups of rats were treated with deltamethrin at concentrations of 0, 30, 300, 1000, 3000 and 6000 ppm (0/0, 2/3, 24/30, 72/84, 241/272 and 425/444 mg/kg/day in males/females, respectively) orally via the diet. Effects were observed as increased mortality, increased incidence of adverse clinical signs (poor condition and neurological effects), decreased body weights and decreased food consumption and water intake. The NOAEL was given as 300 ppm (24/30 mg/kg/day in males/females, respectively). In the second 13-week toxicity study conducted in rats, groups of rats were treated with deltamethrin at doses of 0, 0.1, 1, 2.5 and 10 mg/kg/day via oral gavage. Effects were observed as increased incidence of adverse clinical signs (hypersensitivity) and decreased body weights. The NOAEL was given as 1 and 2.5 mg/kg/day in males and females, respectively.

Two subchronic 13-week toxicity studies were conducted in dogs, both via oral gavage. In the first 13-week toxicity study conducted in dogs, groups of dogs were treated with deltamethrin at doses of 0, 0.1, 1, 2.5 and 10 mg/kg/day via oral gavage. Effects were observed as increased incidence of adverse clinical signs (neurological effects and liquid faeces), decreased body weight gains and decreased food consumption. The NOAEL was given as 1 mg/kg/day. In the second 13-week toxicity study conducted in dogs, groups of dogs were treated with deltamethrin at doses of 0, 2, 10 and 50 mg/kg/day via oral gavage. Effects were observed as increased incidence of adverse clinical signs (neurological signs), decreased body weights and food consumption. The NOAEL was given as 10 mg/kg/day.

Two chronic toxicity studies were conducted in dogs, one for 52 weeks and one for 2 years. In the 52-week toxicity study in dogs, groups of dogs were treated with deltamethrin at doses

of 0, 1, 10 and 50 mg/kg/day via oral gavage. Effects were observed as increased incidence of adverse clinical signs (neurological signs). The NOAEL was given as 1 mg/kg/day. In the 2-year toxicity study in the dog, groups of dogs were treated with deltamethrin at concentrations of 0, 10 and 40 ppm orally via the diet. No adverse effects were observed. The NOAEL was given as 40 ppm (1 mg/kg/day).

Developmental and reproductive toxicity studies

The Draft Renewal Assessment Report on deltamethrin (EC 2017) included eight developmental toxicity studies and one two-generation reproduction study. Developmental toxicity studies were conducted in mice, rats and rabbits.

Two developmental toxicity studies were conducted in mice; in the first study, groups of female mice were treated with deltamethrin at doses of 0, 3, 6 and 12 mg/kg/day via oral gavage on gestation days 7 to 16. Effects were observed as increased incidence of adverse maternal clinical signs (convulsions), decreased maternal body weight and increased incidence of foetal supernumerary ribs. However, insufficient detail was given to determine a NOAEL. In the second study, groups of female mice were treated with deltamethrin at doses of 0, 0.1, 1 and 10 mg/kg/day via oral gavage on gestation days 6 to 17. No maternal or developmental toxicity was observed, therefore, the NOAEL was given as 10 mg/kg/day.

Three developmental toxicity studies were conducted in rats; in the first study, groups of female rats were treated with deltamethrin at doses of 0, 0.1, 1 and 10 mg/kg/day via oral gavage on gestation days 6 to 18. No maternal or developmental toxicity was observed, therefore, the NOAEL was given as 10 mg/kg/day. In the second study, groups of female rats were treated with deltamethrin at doses of 0, 0.1, 1 and 10 mg/kg/day via oral gavage on gestation days 6 to 18. Effects were observed as increased incidence of adverse maternal clinical signs (salivation), decreased maternal bodyweights and decreased neonatal body weights. The maternal NOAEL was given as 2.5 mg/kg/day, the developmental NOAEL was given as 5 mg/kg/day and the neonatal NOAEL was given as < 1.25 mg/kg/day. In the third study, groups of female rats were treated with deltamethrin at doses of 0, 1, 3.3, 7 and 11 mg/kg/day via oral gavage on gestation days 6 to 15. Effects were observed as increased maternal mortality, increased incidence of adverse maternal clinical signs (neurological effects) and decreased maternal body weights. The maternal NOAEL was given as 3.3 mg/kg/day and the developmental NOAEL was given as 11 mg/kg/day.

Three developmental toxicity studies were conducted in rabbits; in the first study, groups of female rabbits were treated with deltamethrin at doses of 0, 10, 25 and 100 mg/kg/day on gestation days 7 to 19. Effects were observed as increased incidence of delayed skeletal ossification in foetuses. The maternal NOAEL was given as 100 mg/kg/day and the developmental NOAEL was given as 25 mg/kg/day. In the second study, groups of female rabbits were treated with deltamethrin at doses of 0, 1, 4 and 16 mg/kg/day on gestation days 6 to 19. No maternal or developmental toxicity was observed, therefore the NOAEL was given as 16 mg/kg/day. In the third study, groups of female rabbits were treated with deltamethrin at doses of 0, 3, 10 and 32 mg/kg/day on gestation days 6 to 28. Effects were

observed on maternal body weight. The maternal NOAEL was given as 10 mg/kg/day and the developmental NOAEL was given as 32 mg/kg/day.

One two-generation reproduction toxicity study was conducted in rats. Groups of rats were treated with doses of 0, 5, 20, 80 and 320 ppm via the diet for two generations. Effects were observed as increased maternal mortality, increased incidence of adverse maternal clinical signs, decreased maternal body weights and food consumption and decreased parental organ weights (ovary, nongravid uterus, pituitary, epididymides and testes) and increased maternal gastric erosions. Effects were also observed as increased pup deaths, decreased lactation index and decreased pup body weights. The reproductive NOAEL was given as 320 ppm (18.3 mg/kg/day), the parental NOAEL was given as 80 ppm (4.2 mg/kg/day) and the offspring NOAEL was given as 80 ppm (7.6 mg/kg/day).

Conclusion: The NOAEL/NOEL selected as the PoD was 1 mg/kg/day, the most conservative NOAEL taken from the 90-day oral gavage repeat-dose toxicity study in rats (EC 2017; Confidential 1977).

Table 3.8 Deltamethrin - Summary of relevant repeat-dose toxicity data

Study type	Test substance	Experimental design	Results	Reference
12-week feeding study in mice	Deltamethrin	Groups of Swiss mice were treated with concentrations of 0, 30, 300, 3000 and 6000 ppm (equivalent to 0/0, 6/8, 62/77, 603/739 and 1318/1391 mg/kg/day in males/females, respectively) orally via the diet for a period of 12 weeks.	Effects observed on mortality, clinical signs, body weight and food consumption. NOAEL given as 62 / 77 mg/kg/day in males/females, respectively.	EC 2017; Confidential (1991)
13-week feeding study in rats	Deltamethrin	Groups of Crl:CD(SD)BR rats were treated with concentrations of 0, 30, 300, 1000, 3000 and 6000 ppm (0/0, 2/3, 24/30, 72/84, 241/272 and 425/444 mg/kg/day in males/females, respectively) orally via the diet for a period of 13 weeks.	Effects observed on mortality, clinical signs, body weight, food consumption and water intake. NOAEL given as 24 / 30 mg/kg/day in males/females, respectively.	EC 2017; Confidential (1991)
13-week oral toxicity study in rats	Deltamethrin	Groups of Sprague-Dawley rats were treated with doses of 0, 0.1, 1, 2.5 and 10 mg/kg/day via oral gavage for a period of 13 weeks.	Effects observed on clinical signs and body weight gain. NOAEL given as 1 mg/kg/day in males and 2.5 mg/kg/day in females.	EC 2017; Confidential (1977)
13-week oral toxicity study in dogs	Deltamethrin	Groups of Beagle dogs were treated with doses of 0, 0.1, 1, 2.5 and 10 mg/kg/day via oral gavage for a period of 13 weeks.	Effects observed on clinical signs, body weight gain and food consumption. NOAEL given as 1 mg/kg/day.	EC 2017; Confidential (1977)
13-week oral toxicity study in dogs	Deltamethrin	Groups of Beagle dogs treated with doses of 0, 2, 10 and 50 mg/kg/day via oral gavage for a period of 13 weeks.	Effects observed on clinical signs, body weight gain and food consumption. NOAEL given as 10 mg/kg/day.	EC 2017; Confidential (1991)
52-week oral toxicity study in dogs	Deltamethrin	Groups of Beagle dogs treated with doses of 0, 1, 10 and 50 mg/kg/day via oral gavage for a period of 52 weeks.	Effects observed on clinical signs. NOAEL given as 1 mg/kg/day.	EC 2017; Confidential (1993)
Two-year chronic toxicity study in dogs	Deltamethrin	Groups of male and female Beagle dogs treated with concentrations of 0, 10 and 40 ppm orally via the diet for periods of up to 2 years.	No adverse effects observed. NOAEL given as 40 ppm (1 mg/kg/day).	EC 2017; Confidential (1980)
Developmental toxicity study in mice	Deltamethrin	Groups of female CD-1 female mice treated with doses of 0, 3, 6 and 12 mg/kg/day via oral gavage on gestation days 7 to 16.	Effects on maternal toxicity (convulsions and decreased body weight) and effects on supernumerary ribs. Insufficient detail to determine NOAELs.	EC 2017; Kavlock et al. (1979)

Study type	Test substance	Experimental design	Results	Reference
Developmental toxicity study in mice	Deltamethrin	Groups of female CD-1 female mice treated with doses of 0, 0.1, 1 and 10 mg/kg/day via oral gavage on gestation days 6 to 17.	No maternal toxicity or developmental toxicity observed. Maternal and developmental NOAEL given as 10 mg/kg/day.	EC 2017; Confidential (1977)
Developmental toxicity study in rats	Deltamethrin	Groups of female Sprague-Dawley rats treated with doses of 0, 0.1, 1 and 10 mg/kg/day via oral gavage on gestation days 6 to 18.	No maternal toxicity or developmental toxicity observed. Maternal and developmental NOAEL given as 10 mg/kg/day.	EC 2017; Confidential (1977)
Developmental toxicity study in rats	Deltamethrin	Groups of female Sprague-Dawley rats treated with doses of 0, 1.25, 2.5 and 5 mg/kg/day via oral gavage on gestation days 7 to 20.	Effects on maternal toxicity (clinical signs and decreased body weights) and effects on decreased neonatal weights. Maternal NOAEL given as 2.5 mg/kg/day and foetal NOAEL given as < 1.25 mg/kg/day.	EC 2017; Kavlock et al. (1979)
Developmental toxicity study in rats	Deltamethrin	Groups of female Sprague-Dawley rats treated with doses of 0, 1, 3.3, 7 and 11 mg/kg/day via oral gavage on gestation days 6 to 15.	Effects on maternal toxicity (mortality, clinical signs and decreased body weights). No developmental toxicity. Maternal NOAEL given as 3.3 mg/kg/day and developmental NOAEL given as 11 mg/kg/day.	EC 2017; Confidential (1990)
Developmental toxicity study in rabbits	Deltamethrin	Groups of female New Zealand White rabbits treated with doses of 0, 10, 25 and 100 mg/kg/day on gestation days 7 to 19.	No maternal toxicity observed. Effects on delayed skeletal ossification. Maternal NOAEL given as >100 mg/kg/day and developmental NOAEL given as 25 mg/kg/day.	EC 2017; Confidential (1990)
Developmental toxicity study in rabbits	Deltamethrin	Groups of female New Zealand White rabbits treated with doses of 0, 1, 4 and 16 mg/kg/day on gestation days 6 to 19.	No maternal toxicity or developmental toxicity observed. Maternal and developmental NOAEL given as 16 mg/kg/day.	EC 2017; Confidential (1977)
Developmental toxicity study in rabbits	Deltamethrin	Groups of female New Zealand White rabbits treated with doses of 0, 3, 10 and 32 mg/kg/day on gestation days 6 to 28.	Effects on maternal toxicity (decreased body weights). No developmental toxicity observed. Maternal NOAEL given as 10	EC 2017; Confidential (2001)

Study type	Test substance	Experimental design	Results	Reference
			mg/kg/day and developmental NOAEL given as 32 mg/kg/day.	
Two-generation reproduction toxicity study in rats	Deltamethrin	Groups of rats treated with doses of 0, 5, 20, 80 and 320 ppm via the diet for two generations.	Effects on parental toxicity (mortality, clinical signs, decreased body weights and food consumption). No effects on reproduction. Effects observed on pup deaths, lactation and pup body weights. Parental NOAEL given as 80 ppm (4.2 mg/kg/day), reproductive NOAEL given as 320 ppm (18.3 mg/kg/day) and offspring NOAEL given as 80 ppm (7.6 mg/kg/day).	EC 2017; Confidential (1992)

3.2.3 DNEL Calculation

All the relevant toxicity studies with deltamethrin were conducted by the oral route of administration, either via dietary inclusion with 24 hour/day *ad libitum* availability or by daily oral gavage administration. Consequently, the PoD for all DNEL calculations was based on an oral NOAEL/NOEL from an appropriate study.

The NOAEL/NOEL selected as the PoD was 1 mg/kg/day, the most conservative NOAEL taken from the 90-day oral gavage repeat-dose toxicity study in rats (EC 2017; Confidential 1977). This study was selected based on the following reasoning:

- The oral gavage route of exposure was preferred over dietary administration of the test substance, as an accurate peak dose exposure was achieved for each individual rat in such studies.
- The 90-day repeat-dose test system is accepted as a good level of predictiveness of the potential exposure associated with the open-water swimming scenario.
- The dose level of 1 mg/kg/day represents the most conservative PoD from other studies of this type (e.g. 52-week and 2-year chronic toxicity studies in dogs by daily oral gavage and dietary administration respectively, and a two-year carcinogenicity study in rats by dietary administration).
- There were no carcinogenicity effects at dietary inclusion levels equivalent to >30 mg/kg/day, and there were negative results for mutagenicity/clastogenicity in *in vitro* assays.
- The most conservative NOAEL in rat developmental toxicity studies also falls within this range (i.e. 1.25 mg/kg/day by oral gavage).

Under other regulations, alternative health based exposure limits have been derived. This specific PoD of 1 mg/kg/day has also been used by the World Health Organisation (WHO) and the Joint Expert Committee on Food Additives (JECFA – administered jointly by WHO and FAO (The Food and Agriculture Organisation of the United Nations)) to determine the ADI of 0.01 mg/kg bw for deltamethrin (WHO 2003).

The general formula for the calculation of an Endpoint-specific DNEL is as follows:

- $DNEL_{Endpoint\ specific} = NOAEL_{corrected}/AF_1 \times AF_2 \times AF_3 \times AF_4 \times AF_5$

Where;
$NOAEL_{corrected}$ is the PoD corrected for the appropriate route of administration and any known differences in absorption kinetics.
AF are the various Assessment Factors (see below).

Oral

Selection of relevant dose-descriptor: PoD 1 mg/kg/day (as indicated above)

Modification of relevant dose-descriptor: Dose descriptor modification is not considered appropriate in this case since the human exposure route is the same as that in experimental animals and the experimental exposure conditions are considered to be similar to the swallowing of contaminated water by an open water swimmer.

Since the start and end route of exposure are the same and no specific absorption data are available, it will be assumed that there is 100% absorption as the worst-case-scenario default.

Application of assessment factors:

- Interspecies differences - apply a factor for allometric scaling (4 for rat) for $oral_{rat}$ to $oral_{human}$ exposure.
- Intraspecies differences – use Worker default of 5 as this will replicate short term exposure in a light-work scenario (open water swimmer).
- Differences in duration of exposure - PoD taken from a subacute toxicity study (90-days duration) and this daily exposure is considered representative of the frequency of exposure in open water swimming. Therefore, AF is 1.
- Issues related to dose response – PoD based on NOAEL. Deltamethrin is classified as Acute Tox 3; H301 – Harmful if swallowed and Acute Tox 3; H331 – Toxic if inhaled, therefore, an AF of 2 is used to account for this local toxicity.
- Quality of whole database – several oral toxicity studies in rodents are available for evaluation including the preferred route of administration of oral gavage. All of the studies listed in Tables 3.7 and 3.8 are of good quality and reliability, as indicated by the Klimisch scores (reported where available). Consequently, the standard default assessment factor of 1 should be applied.
- Therefore;
- $DNEL_{Oral}$ = $NOAEL_{corrected}$/AF1 x AF2 x AF3 x AF4 x AF5

  = 1/4 x 5 x 1 x 2 x 1

  = **0.025 mg/kg/day**

Dermal

Selection of relevant dose-descriptor: PoD 1 mg/kg/day (as indicated above)

Modification of relevant dose-descriptor: Convert rat oral NOAEL dose-descriptor into dermal rat NOAEL by correcting for differences in absorption between the two routes. As there is no specific absorption data available by these routes of administration, it will be assumed that there is 100% absorption as the worst-case scenario and no modification of the dose-descriptor is required.

Application of assessment factors:

- Interspecies differences – apply a factor for allometric scaling (4 for rat) for $oral_{rat}$ to $dermal_{human}$ exposure.

- Intraspecies differences - use Worker default of 5 as this will replicate short term exposure in a light-work scenario (open water swimmer).

- Differences in duration of exposure – PoD taken from a subacute toxicity study (90-days duration) and this daily exposure is considered representative of the frequency of exposure in open water swimming. Therefore, AF is 1.

- Issues related to dose response - PoD based on NOAEL. There were no specific toxicities in mammalian studies associated with the specific mode of action of depolarising Na+ channels and there were no local toxicity classifications, therefore, the AF is 1.

- Quality of whole database – several oral toxicity studies in rodents are available for evaluation including the preferred route of administration of oral gavage. All of the studies listed in Tables 3.7 and 3.8 are of good quality and reliability, as indicated by the Klimisch scores (reported where available). Consequently, the standard default assessment factor of 1 should be applied.

Therefore;

- $DNEL_{Dermal}$ = $NOAEL_{corrected}$/AF1 x AF2 x AF3 x AF4 x AF5

  = 1/4 x 5 x 1 x 1 x 1

  = **0.05 mg/kg/day**

3.3 Hydrogen peroxide

3.3.1 Hazard assessment

3.3.1.1 Regulatory data

Hydrogen peroxide is a registered chemical under REACH[7], therefore, relevant toxicological data is available from the ECHA registration dosser. Additionally, a Cosmetics Ingredient Review was conducted for hydrogen peroxide in 2018, and relevant toxicological data is also available from this report. Relevant data has been extracted from these sources.

3.3.1.2 Literature search

The search was conducted using the substance name and identifiers, with an appropriate search string and the results of the search are summarised in Table 3.9. The literature search was conducted from 2018 as a Cosmetics Ingredient Review was conducted in 2018 which had searched relevant databases for toxicological information.

Table 3.9 Hydrogen peroxide - Literature search results

Search	Number of hits		Combined number of hits after duplicates removed
	Web of Science	PubMed	
("Hydrogen peroxide") AND (Toxicology OR toxicity OR Chronic OR Subchronic OR Sublethal OR NOAEL OR LOAEL OR Repro* OR Carcinogen* OR Mutagen* OR Genotox* OR Oral OR Dermal OR Inhal* OR Pharmacokinetic OR Toxicokinetic) AND ("Rat" OR Mouse OR Human OR Dog OR Rabbit OR "In vitro")	2818	1535	3655

Where references were identified, they were downloaded into an Excel spreadsheet as a record of the searches. The titles and abstracts of the articles were screened for potentially relevant articles. No relevant information was identified through the screening process, so no additional relevant toxicology information was found for the target substance.

3.3.2 Review of toxicological data

3.3.2.1 Acute and local toxicity

Oral toxicity

The acute oral toxicity of hydrogen peroxide has been tested in a number of studies. In one study, rats were exposed to a 70% solution of hydrogen peroxide resulted in LC_{50} values slightly above 1000 mg/kg in male and below 700 mg/kg in female animals, which triggers

[7] https://echa.europa.eu/registration-dossier/-/registered-dossier/15701/1/1

the CLP classification with Acute Toxicity Hazard Category 4. A study with rats exposed to a 35% solution of hydrogen peroxide, demonstrates that a similar classification is required for a 35% solution of hydrogen peroxide. The current concentration limit for the classification of hydrogen peroxide solutions as harmful if swallowed according to the Directive 67/548/EEC is ≥8% w/w. In conclusion, the hydrogen peroxide is classified for acute oral toxicity in solutions containing 8% or more of the substance.

Dermal toxicity

The acute dermal toxicity of hydrogen peroxide has been tested. The available acute dermal toxicity studies performed with 35% and 70% solutions clearly demonstrate that no classification for acute dermal toxicity is required.

The skin corrosion and irritation potential of hydrogen peroxide have been tested. The application of 35% hydrogen peroxide causes slight to moderate, transient irritation and the current classification as "Skin irritant category 2" (H315) according to Regulation (EC) No 1272/2008 is supported by this finding. Hydrogen peroxide solutions ranging from 50 to 70% are corrosive to the skin and fall into the "Skin corrosion category 1B" (H314). Solutions containing more than 70 % are very corrosive to the skin and fall into the "Skin corrosion category 1A" (H314).

In conclusion, hydrogen peroxide is classified for skin corrosion in solutions containing >50% of the substance and is classified for skin irritation in solutions containing 35 – 50% of the substance. The EU Risk Assessment Report for hydrogen peroxide concludes that the substance should not be classified as skin sensitiser (ECB 2003).

Inhalatory toxicity

The acute inhalation toxicity of hydrogen peroxide has been tested in a number of studies. The studies were performed with aerosols generated from 50% and 70% hydrogen peroxide solutions in rats. Mortality was observed with the 70% solution. The current concentration limit for the classification of hydrogen peroxide solutions as harmful by inhalation according to the Directive 67/548/EEC is ≥50%, due to skin corrosion classifications. In conclusion, hydrogen peroxide is classified for acute inhalation toxicity in solutions containing 50% or more of the substance.

Respiratory tract irritation of hydrogen peroxide has also been assessed in studies with 50% solutions of hydrogen peroxide and therefore hydrogen peroxide is also classified for respiratory tract irritation.

Table 3.10 Hydrogen peroxide classifications

Endpoint	Classification
Acute toxicity – oral route	Acute Tox 4; H302 – Harmful if swallowed
Skin corrosion / irritation	Skin Corr. 1A; H314: Causes severe skin burns and eye damage (>70%)
Skin corrosion / irritation	Skin Corr. 1B; H314: Causes severe skin burns and eye damage (50 – 70%)

Endpoint	Classification
Skin corrosion / irritation	Skin Irrit. 2; H315: Causes skin irritation (<50%)
Acute toxicity - inhalation route	Acute Tox 4; H332 – Toxic if inhaled
Respiratory tract irritation	STOT Single Exp. 3; H335: May cause respiratory irritation

Conclusion: As hydrogen peroxide is classified for acute oral, skin corrosion and acute and irritation inhalatory toxicity, the dose-response assessment factor of the DNEL calculation has been adjusted to incorporate this local toxicity risk into the systemic DNEL.

3.3.2.2 Genotoxicity and carcinogenicity

Details of relevant studies are presented in Table 3.11. The outcome of any reliability assessments conducted on the studies listed is unknown and there was no opportunity to conduct *de novo* Klimisch assessments as the original data were not available.

A number of genotoxicity tests have been conducted with hydrogen peroxide. Gene mutation tests in bacteria, including Ames tests, were conducted with hydrogen peroxide (CIR 2018). In the review, a total of 16 Ames tests were reported with hydrogen peroxide and inconsistent results were obtained from the assays. An additional 9 bacterial mutation assays were also reported, with varying study designs in bacterial forward mutation assays. In the Ames tests, positive results in at least one strain were reported in 14 / 16 assays, with the most common response being an increase in the number of revertant colonies in *Salmonella typhimurium* strains without metabolic activation (starting at 33 µg/plate). However, the results between strains were extremely inconsistent as in one assay with metabolic activation, Hydrogen Peroxide was mutagenic in strain TA100, but not in TA98, TA1535, TA1537, and TA1538 and in another, Hydrogen Peroxide without metabolic activation was weakly mutagenic in strain TA102, but less genotoxic with metabolic activation (CIR 2018). In the non-Ames assays, hydrogen peroxide was mutagenic in *E. coli* K12 kat(-) and kat(+) strains, *B. subtilis* and *S. typhimurium*.

Hydrogen peroxide was assessed for *in vitro* cytogenicity and chromosome aberration in a number of assays. An EU risk assessment report was published in 2003 (ECB 2003) and identified ten different studies assessing the chromosomal aberration, micronucleus induction and chromatid translocations of hydrogen peroxide in a number of cell lines (CHO, CHL, CHC, V79, mouse skin cells and splenocytes, human embryonic fibroblasts, D98/AH2 (variant of HeLa) cells). The range of concentrations tested was from 0.83 µM to 7.35 mM (28.1 ng/mL to 0.25 mg/mL), without metabolic activation. Only one study (330 mM to 3.3 M) included metabolic activation. Positive results were observed in 8 / 10 studies, indicating that hydrogen peroxide has the potential to induce chromosomal aberrations in mammalian cells without metabolic activation.

Hydrogen peroxide was also assessed for *in vitro* gene mutation in a number of assays. The EU risk assessment report (ECB 2003) reported eleven different studies assessing the gene mutation in mammalian cells in a number of cell lines (CHO, V79, CV-1, and L5178Y) using

different endpoints (HGPRT, thymidine kinase, 6-thioguanine resistance, 6-azaguanidine resistance, ouabain resistance, mutation of the supF locus of the pZ189 plasmid). The range of concentrations tested was from 0.2 µM to 10 mM. Positive results were observed in 7 / 11 studies, negative results were observed in 3 / 11 studies and one study produced an ambiguous result. These studies indicate that hydrogen peroxide has the potential to induce mutations in mammalian cells without metabolic activation.

Additionally, DNA damage was assessed *in vivo* using Comet Assays and hydrogen peroxide was mutagenic in mouse lymphoma cells, rat hepatocytes*, S. cerevisiae*, V79 cells, MCF-7 and MCF-10A breast cancer cells, human lymphocytes, human fibroblasts, HeLa cells and HEP G2 cells (CIR 2018).

The *in vivo* genotoxicity of hydrogen peroxide has been assessed in four studies, conducted in rats and mice. Two mouse micronucleus studies were conducted, in the first, groups of 5 male and 5 female Swiss OF1/ICO:OF1 mice treated with hydrogen peroxide via a single intraperitoneal injection at doses of 0, 250, 500, 1000 mg/kg. Mice were killed at 24 or 48 hours and cytogenetic damage in bone marrow was evaluated. Under the experimental conditions, hydrogen peroxide did not induce cytogenetic damage in the bone marrow cells at any dose level. In the second study, groups of 10 male and 10 female C57BL/6NCr1BR mice treated with hydrogen peroxide orally via drinking water at doses of 0, 200, 1000, 3000 or 6000 ppm (males: 0, 42.4, 164, 415 or 536 mg/kg/day; females: 0, 48.5, 198, 485 or 774 mg/kg/day) for a period of 2 weeks. Cytogenetic damage in bone marrow was evaluated. No statistically significant increase in the frequency of micronucleated polychromatic erythrocytes or decrease in the ratio of polychromatic/normochromatic erythrocytes observed. Two additional *in vivo* genotoxicity studies were conducted, an *in vivo - in vitro* hepatocyte unscheduled DNA synthesis test in rats and a dermal genotoxicity assay in mice. Negative results were observed in both studies.

Although hydrogen peroxide produced numerous *in vitro* positive genotoxicity results, negative results for genotoxicity were observed in all *in vivo* genotoxicity assays conducted. Therefore, the available studies indicate that hydrogen peroxide is not genotoxic or mutagenic under *in vivo* conditions, and the substance is not classified for mutagenicity (ECB 2003).

A number of carcinogenicity studies have been conducted with hydrogen peroxide in mice and rats, through the oral and dermal routes of exposure.

A total of six oral carcinogenicity studies (Table 3.8) were identified from a Cosmetics Ingredient Review (CIR 2018) and the EU risk assessment (ECB 2003). Five of the studies were conducted in mice, and one conducted in rats, and all were exposed via the drinking water.

In the studies conducted in mice, increased incidences of duodenal hyperplasia and localised duodenal carcinomas were observed, as well as stomach lesions and tumours. In the comparable study with rats, hydrogen peroxide produced no carcinogenic effect, and no tumours were observed in the gastrointestinal tract. A number of tumour promotion studies

have also been conducted, in which hydrogen peroxide demonstrated a promoting effect in rat intestinal carcinogenesis and Syrian hamster buccal pouch carcinogenesis (ECB 2003).

In a review conducted by DeSesso et al. (2000), the tumorigenic effects observed in mice were reviewed in context. The review discussed the increased incidences of duodenal tumours in mice receiving hydrogen peroxide via the drinking water. It states that due to the chemistry of dilute hydrogen peroxide solutions and the anatomy of the gastrointestinal tract of mice, the increased incidence of tumours is most likely related to the decreased water consumption of the mice, causing abrasion of the luminal lining from the pelleted rodent food. The review concludes that oral ingestion of hydrogen peroxide should not be considered a carcinogenic threat (DeSesso et al. 2000).

Dermal carcinogenicity studies were also conducted in mice and rats and was not carcinogenic when dermally administered to mice and rats (CIR 2018). However, the dermal studies were not sufficient to evaluate carcinogenicity as they were applied as hair dye formulations, rather than as hydrogen peroxide alone.

The conclusion from the EU risk assessment for hydrogen peroxide (ECB 2003), stated that "due to the specific mechanism of carcinogenicity exhibited by hydrogen peroxide, there is doubt regarding the practical significance of the carcinogenicity of hydrogen peroxide."

Additionally, the International Agency for Research on Cancer (IARC) concluded that there is inadequate evidence in humans for the carcinogenicity of hydrogen peroxide and limited evidence in experimental animals for the carcinogenicity of hydrogen peroxide (IARC 1999). The overall evaluation of hydrogen peroxide was that it was not classifiable as carcinogenic to humans and placed in Group 3 (IARC 1999).

Conclusion: As hydrogen peroxide is not classified for mutagenicity or carcinogenicity, no specific adjustments for this endpoint are required to the assessment factors in the systemic DNEL derivation.

Table 3.11 Hydrogen peroxide - Summary of relevant genetic/carcinogenic toxicity data

Study type	Test substance	Experimental design	Results	Reference
Mammalian erythrocyte micronucleus test (OECD 474)	Hydrogen peroxide	Groups of 5 male and 5 female Swiss OF1/ICO:OF1 mice treated with hydrogen peroxide via a single intraperitoneal injection at doses of 0, 250, 500, 1000 mg/kg. Mice were killed at 24 or 48 h and cytogenetic damage in bone marrow was evaluated.	Negative. Hydrogen peroxide did not induce cytogenetic damage in bone marrow cells of mice when administered.	ECHA registration dossier; CEFIC (1995); Klimisch 1
Mammalian erythrocyte micronucleus test (OECD 474)	Hydrogen peroxide	Groups of 10 male and 10 female C57BL/6NCr1BR mice treated with hydrogen peroxide orally via drinking water at doses of 0, 200, 1000, 3000 or 6000 ppm (males: 0, 42.4, 164, 415 or 536 mg/kg/day; females: 0, 48.5, 198, 485 or 774 mg/kg/day) for a period of 2 weeks. Cytogenetic damage in bone marrow was evaluated.	Negative. Hydrogen peroxide did not show any genotoxic effects at tested concentrations.	ECHA registration dossier; DuPont (1995); Klimisch 1
In vivo - in vitro hepatocyte unscheduled DNA synthesis (UDS) (OECD 486)	Hydrogen peroxide	Groups of 5 to 6 male Wistar rats treated with hydrogen peroxide via a single intravenous injection at doses of 0, 25, or 50 mg/kg. Rats were then killed at 2 - 4 h or 12 -14 h. Hepatocytes were treated with 3H-thymidine and put onto slides and examined for unscheduled DNA synthesis.	Negative. Hydrogen Peroxide did not induce unscheduled DNA synthesis following treatment in vivo.	ECHA registration dossier; CEFIC (1997); Klimisch 2
Dermal genotoxicity assay in mice	Hydrogen peroxide	Groups of 10 female Sencar mice treated with hydrogen peroxide via dermal application at loading rates of 10, 100 and 200 mmol in 200 ml ethanol (0.2, 1.6 and 3.2%) for a period of 4 weeks. Application sites were examined for DNA damage.	Negative for genotoxicity.	CIR (2018)
Carcinogenicity study in mice	Hydrogen peroxide	Groups of male and female C57BL/6J mice treated with hydrogen peroxide orally via the drinking water at concentrations of 0, 0.1 and 0.4% for a period of 100 weeks.	Increased incidence of localised duodenal carcinomas in high dose group. LOEL estimated as 0.1%.	CIR (2018); Ito et al. 1981
Carcinogenicity study in mice	Hydrogen peroxide	Groups of male and female C57BL, DBA/2N and BALB/cAnN mice treated with hydrogen peroxide orally via the drinking water at concentrations of 0, 0.1 and 0.4% for periods of up to 104 weeks.	Only stomach and duodenum studied. Increased incidence of duodenal carcinomas at low and high dose. LOEL estimated as 0.1%.	ECB (2003); Ito et al. 1982
Carcinogenicity study in mice	Hydrogen peroxide	Groups of male and female C57BL/6N mice treated with hydrogen peroxide orally via the drinking water at a concentration of 0.4%. Mice were killed and necropsied at	Nodules (hyperplastic lesions, adenomas, and carcinomas) were observed in both duodenum and	CIR (2018); Ito et al. 1982

Study type	Test substance	Experimental design	Results	Reference
		30-day intervals up to 210 days, and then every 60, 70 or 90 days up to 630 days. Reversibility of lesions was investigated in groups of mice treated with Hydrogen Peroxide (0.4%) for 120, 140, 150 or 180 days followed by a treatment-free period of 10 to 30 days.	stomach from 90 days until end of experiment. During recovery period, stomach lesions regressed completely, but some duodenal lesions persisted.	
Carcinogenicity study in mice	Hydrogen peroxide	Groups of male and female DBA/2N, BALB/cAnN and C57BL/6N mice treated with hydrogen peroxide orally via the drinking water at a concentration of 0.4% for a period of 90 to 210 days.	Increased incidence of gastric and duodenal nodules in all species treated with hydrogen peroxide.	CIR (2018); 51
Carcinogenicity study in mice	Hydrogen peroxide	Groups of male and female C3H/HeN, B6C3F1, C57BL/6N and C3H/Cbs mice treated with hydrogen peroxide orally via the drinking water at a concentration of 0.4% for a period of 6 to 7 months.	Increased incidence of duodenal nodules (hyperplastic lesions, adenomas and carcinomas) in all species treated with hydrogen peroxide.	CIR (2018); Ito et al. 1984
Carcinogenicity study in rats	Hydrogen peroxide	Groups of 50 male and 50 female Fischer F433 rats treated with hydrogen peroxide orally via the drinking water at concentrations of 0, 0.3 and 0.6% for a period of 78 weeks, followed by a 6-month recovery period.	Effects observed on body weights. No carcinogenic effects observed.	CIR (2018); Takayama et al. 1980

3.3.2.3 Repeat dose toxicity

Details of relevant studies are presented in Table 3.12. The outcome of any reliability assessments conducted on the studies listed is unknown and there was no opportunity to conduct *de novo* Klimisch assessments as the original data were not available.

A number of relevant repeat dose toxicity studies have been conducted using hydrogen peroxide, via the oral, dermal and inhalation routes of exposure. However, many of these studies have severe limitations in study design and reporting of results, as they are non-guideline literature studies rather than regulatory studies. All relevant studies conducted with hydrogen peroxide are tabulated in Table 3.12, with the studies with limitations in study design and reporting of results indicated in the table.

Two relevant, repeat-dose, regulatory guideline studies have been conducted with hydrogen peroxide, one via the inhalation route of exposure and one via the oral route of exposure.

In the 28-day repeat dose inhalation toxicity study, groups of 5 male and 5 female Alpk:APfSD rats were treated with hydrogen peroxide via whole body inhalation exposure at doses of 0 (control), 2.9, 14.6 or 33 mg/m^3. Exposures were conducted for 6 hours daily, 5 days per week for 28 days (ECB 2003). Increased clinical signs associated with respiratory tract irritation were observed at 14.6 and 33 mg/m^3, but not at 2.9 mg/m^3. Histopathological lesions were also observed at 14.6 and 33 mg/m^3, including concentration related necrosis and inflammation of the epithelium in the anterior regions of the nasal cavity and mononuclear cell infiltration in the larynx. In the lungs, one male rat in each exposure group and two female rats treated with 33 mg/m^3 showed perivascular neutrophil infiltration and haemorrhage was observed in some animals at the two lower dose levels. Similar lesions were not observed in the control animals. Regarding the pathology in the lungs, the authors of the study considered it unlikely that the effects were treatment related due to the absence of a relationship with exposure concentration and the low incidence, and hence the NOAEL of the study was given as 2.9 mg/m^3 (ECB 2003).

In the 90-day oral toxicity study in rats, groups of 15 male and 15 female C57BL/6NCrl BR mice were treated with hydrogen peroxide orally via the drinking water at concentrations of 0 (control), 100, 300, 1000 or 3000 ppm (equivalent to 0/0, 26/37, 76/103, 239/328 or 547/785 mg/kg/day in males/females, respectively) for a period of 90 days. Additionally, 5 mice/sex/group continued on untreated distilled water for additional 6 weeks (recovery period). Effects were observed at 300 ppm and above on decreased body weights and food consumption. Histopathological lesions were observed in in the duodenum at terminal sacrifice, including, an increase in cross sectional diameter and a larger mucosal area with broader, more substantial villi when compared to those of control mice. Based on these effects, the NOAEL was given as 100 ppm (26 and 37 mg/kg/day for males and females, respectively) (ECB 2003).

Conclusion: The NOAEL/NOEL selected as the Point of Departure (PoD) for DNEL derivation was 20 mg/kg/day, the most conservative NOAEL selected from the 100-day repeat-dose toxicity study in rats by daily oral gavage (Kawasaki 1969).

Table 3.12 Hydrogen peroxide - Summary of relevant repeat-dose toxicity data

Study type	Test substance	Experimental design	Results	Reference
6-week inhalation toxicity study in rats*	Hydrogen peroxide vapour	A single group of 23 rats were treated with hydrogen peroxide via whole body inhalation exposure at a dose of 93 mg/m^3 (67 ppm). Exposures conducted for 6 hours daily, 5 days per week for 6 weeks (180 hours total).	Effects observed on clinical signs (nasal discharge, oedematous feet, irritation of skin in the groin region and hair loss). LOAEL given as 93 mg/m^3.	ECB 2003; Comstock et al. (1954) and Oberst et al. (1954)
6-week inhalation toxicity study in mice*	Hydrogen peroxide vapour	Groups of 10 mice were treated with hydrogen peroxide via whole body inhalation exposure at doses of 79 or 107 mg/m^3 (57 or 77 ppm). Exposures conducted for 6 hours daily, 5 days per week for 6 weeks (180 hours total).	Effects observed on clinical signs (nasal discharge, oedematous feet, irritation of skin in the groin region and hair loss). LOAEL given as 79 mg/m^3.	ECB 2003; Comstock et al. (1954) and Oberst et al. (1954)
6-month inhalation toxicity study in dogs*	Hydrogen peroxide vapour	A single group of 2 dogs were treated with hydrogen peroxide via whole body inhalation exposure at a dose of 10 mg/m^3 (7 ppm). Exposures conducted for 6 hours daily, 5 days per week for 6 months (126 exposures total).	Effects observed on clinical signs (sneezing, lacrimation, external skin irritation, hair bleaching, hair loss and skin thickening) and lung lesions. LOAEL given as 10 mg/m^3.	ECB 2003; Comstock et al. (1954) and Oberst et al. (1954)
28-day inhalation toxicity study in rats (OECD 412)	Hydrogen peroxide vapour	Groups of 5 male and 5 female Alpk:APfSD rats were treated with hydrogen peroxide via whole body inhalation exposure at doses of 0 (control), 2.9, 14.6 or 33 mg/m^3. Exposures conducted for 6 hours daily, 5 days per week for 28 days.	Effects observed on respiratory tract irritation and nasal lesions (necrosis and inflammation of the epithelium). NOAEL given as 2.9 mg/m^3.	ECB 2003; CEFIC Peroxygen Sector Group (2002)
4-month inhalation study in rats*	Hydrogen peroxide vapour	Groups of rats were treated with hydrogen peroxide via whole body inhalation exposure at doses of 0.1 to 10.0 mg/m^3 for up to 4 months.	Effects observed on clinical chemistry parameters (serum epoxidase, pulmonary SDH, MAO, acid phosphatase, diesterase). NOEL given as 1 mg/m^3.	ECB 2003; Kondrashov (1977)
12-week oral toxicity study in rats	Hydrogen peroxide (5% solution)	Groups of 12 male Wistar-JCL rats were treated with hydrogen peroxide orally via stomach tube at doses of 0 (control), 56.2, 168.7 or 506 mg/kg/day for 12 weeks.	Effects observed on decreased body weights, clinical chemistry parameters and gastric lesions. Effects at all doses, therefore the LOEL was given as 56.2 mg/kg/day.	ECB 2003; Ito et al. (1976)
100-day oral toxicity study in rats	Hydrogen peroxide (0.06 - 6% solution)	Groups of 9 – 12 male Wistar rats were treated with hydrogen peroxide orally via stomach tube at doses of 0 (control), 6, 10, 20, 30 or 60 mg/kg/day for periods of up to 100 days.	Effects observed on decreased body weights, increased spleen weights and alterations in clinical chemistry parameters (haematocrit, plasma	ECB 2003; Kawasaki et al. (1969)

Study type	Test substance	Experimental design	Results	Reference
			protein and catalase). NOAEL given as 20 mg/kg/day.	
6-month oral toxicity study in rats*	Hydrogen peroxide (0.0001 – 0.1%)	Groups of male and female rats were treated with hydrogen peroxide orally via stomach tube at doses of 0.005 to 50 mg/kg/day for up to 6 months.	Effects observed on decreased body weights, haematology and clinical chemistry parameters and gastrointestinal lesions. NOEL given as 0.005 mg/kg/day.	ECB 2003; Antonova et al. (1974)
90-day oral toxicity study in rats*	Hydrogen peroxide	Groups of 6 male Wistar rats were treated with hydrogen peroxide orally via the diet at doses of 0, 0.6, 1, 3 or 6 mg/20g feed/day for a period of 90 days.	No effects reported. Hydrogen peroxide likely rapidly degraded in the feed therefore exposure is uncertain. NOAEL given as 6 mg/20g feed/day.	ECB 2003; Kawasaki et al. (1969)
8-week oral toxicity study in rats*	Hydrogen peroxide	Groups of male Holzman rats were treated with hydrogen peroxide orally via the drinking water at concentrations of 0, 0.5, 1 or 1.5% (study I) and 0, 1 or 1.5% (study II) for up to 8 weeks.	Effects observed on decreased body weights, increased mortality and periodontial lesions. Effects at all doses, therefore the LOEL was given as 0.5%.	ECB 2003; Shapiro et al. (1960)
56-day oral toxicity study in rats*	Hydrogen peroxide	A single group of 8 male Wistar rats were treated with hydrogen peroxide orally via the drinking water at a concentration of 0.5% for a period of 56 days.	Effects observed on decreased water consumption and body weights and clinical chemistry parameters (Se-dependent glutathione peroxidase and catalase). Effects at single dose tested, therefore the LOEL was given as 0.5%.	ECB 2003; Kihlström et al. (1986)
40-day oral toxicity study in mice*	Hydrogen peroxide	A single group of 8 NMR1 mice were treated with hydrogen peroxide orally via the drinking water at a concentration of 0.5% for a period of 40 days.	Effects observed on decreased water intake and body weights. Effects at single dose tested, therefore the LOEL was given as 0.5%.	ECB 2003; Kihlström et al. (1986)
3-week oral toxicity study in rats*	Hydrogen peroxide	A single group of male Osborne-Mendel rats were treated with hydrogen peroxide orally via the drinking water at a concentration of 0.45% for a period of 3 weeks.	Effects observed on decreased water intake and body weights.	ECB 2003; Hankin (1958)
10-week oral toxicity study in rats	Hydrogen peroxide	Groups of 10 male and 10 female F344 rats were treated with hydrogen peroxide orally via the drinking water at concentrations of 0 (control), 0.15, 0.3, 0.6, 1.2 or 2.4% for up to 10 weeks.	Effects observed on decreased body weight gains, gastric erosions and ulcer and testicular lesions. Effects at all doses, therefore the LOEL was given as 0.15%.	ECB 2003; Takayama (1980)

Study type	Test substance	Experimental design	Results	Reference
290-day oral toxicity study in rats*	Hydrogen peroxide	Groups of male rats were treated with hydrogen peroxide orally via the drinking water at concentrations of 0 (control), 0.25, 0.5, 2.5, 5 or 10% for up to 290 days.	Effects observed on mortality and decreased body weights. LOEL given as 0.5%.	ECB 2003; Romanowski et al. (1960)
35-week oral toxicity study in mice*	Hydrogen peroxide	Groups of 4 male mice treated with treated with hydrogen peroxide orally via the drinking water at concentrations of 0 (control) or 0.15% for up to 35 weeks.	Effects observed on liver, gastric mucosa, kidney, spleen and small intestine lesions. Effects at single dose tested, therefore the LOEL was given as 0.15%.	ECB 2003; Aoki and Tani (1972)
14-day oral toxicity study in mice	Hydrogen peroxide	Groups of 10 male and 10 female C57BL/6NCr1BR mice were treated with hydrogen peroxide orally via the drinking water at concentrations of 0 (control), 200, 1000, 3000 or 6000 ppm (equivalent to 0/0, 42.4/48.5, 164/198, 415/485 or 536/774 mg/kg/day in males/females, respectively) for a period of 14 days.	Effects observed on decreased body weights and water intake and lesions in the stomach and duodenum. NOAEL given as 1000 ppm (164/198 mg/kg/day in males/females, respectively).	ECB 2003; DuPont (1995)
90-day oral toxicity study in mice (OECD 408)	Hydrogen peroxide	Groups of 15 male and 15 female C57BL/6NCrl BR mice were treated with hydrogen peroxide orally via the drinking water at concentrations of 0 (control), 100, 300, 1000 or 3000 ppm (equivalent to 0/0, 26/37, 76/103, 239/328 or 547/785 mg/kg/day in males/females, respectively) for a period of 90 days. 5 mice/sex/group continued on untreated distilled water for additional 6 weeks (recovery period).	Effects observed on decreased body weight and food consumption, haematology parameters (total protein and globulin) and duodenal lesions. NOAEL given as 100 ppm (26/37 mg/kg/day in males/females, respectively).	ECB 2003; FMC (1997)
Developmental toxicity study in mice*	Hydrogen peroxide	Groups of male mice were treated with hydrogen peroxide orally via the drinking water at concentrations of 0.33, 1 or 3% for 7, 21, or 28 days premating. Female mice were then treated with of 0.33, 1 or 3% following mating.	No effects observed on reproduction or spermatozoa abnormalities. Severe maternal toxicity observed. No NOAEL could be determined due to limitations in study design (no controls).	ECB 2003; Wales et al. (1959)
Developmental toxicity study in rabbits*	Hydrogen peroxide	Groups of male rabbits were treated with hydrogen peroxide orally via the drinking water at concentrations of 0.33, 1 or 3% for 7, 21, or 28 days premating. Female mice were then treated with of 0.33, 1 or 3% following mating.	No effects observed on reproduction or spermatozoa abnormalities. Severe maternal toxicity observed. No NOAEL could be determined due to limitations in study design (no controls).	ECB 2003; Wales et al. (1959)

* Limitations in study design or reporting (ECB 2003).

* Limitations in study design or reporting (ECB 2003).

3.3.3 DNEL Calculation

Relevant toxicity studies with hydrogen peroxide were conducted by the oral route of administration, either via inclusion in the drinking water with 24 hour/day *ad libitum* availability or by daily oral gavage administration, and also via repeated daily whole-body inhalation exposure. Consequently, the PoD for oral and dermal DNEL calculations were based on an oral NOAEL/NOEL from an appropriate study.

The NOAEL/NOEL selected as the PoD for DNEL derivation was 20 mg/kg/day, the most conservative NOAEL selected from the 100-day repeat-dose toxicity study in rats by daily oral gavage (Kawasaki 1969). This study was selected based on the following reasoning:

- Oral gavage was preferred over drinking water exposure because an accurate peak dose exposure was achieved for each individual rat.
- The 100-day repeat-dose test system is accepted as good level of predictiveness of the potential exposure associated with the open-water swimming scenario.
- The dose level of 20 mg/kg/day represents the most conservative oral PoD from other studies of this type (e.g. 90-day toxicity study in mice via the drinking water, 1997).
- There were no carcinogenicity effects observed in a drinking water study in rats at up to 0.6% concentration. Indications of local tumorigenicity in the gastro-intestinal tract of mice were observed at drinking water inclusion levels >0.1%.
- It is not possible to assess how NOAEL from developmental toxicity studies relates to the NOAEL in non-pregnant rats.

Under other regulations, alternative health based exposure limits have been derived. In the EU risk assessment report (ECB 2003), a NOAEL of 26-37 mg/kg/day taken from the 90-day drinking water study in mice (1997) was used as the PoD for long-term oral ADI derivation.

The general formula for the calculation of an Endpoint-specific DNEL is as follows:

- $DNEL_{Endpoint\ specific} = NOAEL_{corrected}/AF_1 \times AF_2 \times AF_3 \times AF_4 \times AF_5$

Where;
$NOAEL_{corrected}$ is the PoD corrected for the appropriate route of administration and any known differences in absorption kinetics.
AF are the various Assessment Factors (see below).

Oral

Selection of relevant dose-descriptor: PoD 20 mg/kg/day (as indicated above)

Modification of relevant dose-descriptor: Dose descriptor modification is not considered appropriate in this case since the human exposure route is the same as that in experimental

animals and the experimental exposure conditions are considered to be similar to the swallowing of contaminated water by an open water swimmer.

Since the start and end route of exposure are the same and no specific absorption data are available, it will be assumed that there is 100% absorption as the worst-case-scenario default.

Application of assessment factors:

- Interspecies differences - apply a factor for allometric scaling (4 for rat) for $oral_{rat}$ to $oral_{human}$ exposure
- Intraspecies differences – use Worker default of 5 as this will replicate short term exposure in a light-work scenario (open water swimmer).
- Differences in duration of exposure - PoD taken from a subacute toxicity study (100-days duration) and this daily exposure is considered representative of the frequency of exposure in open water swimming. Therefore, AF is 1.
- Issues related to dose response – PoD based on NOAEL. Hydrogen peroxide is classified as Acute Tox 4; H302 – Harmful if swallowed and Acute Tox 4; H332 – Toxic if inhaled, therefore, an AF of 2 is used to account for this local toxicity.
- Quality of whole database – several oral toxicity studies in rodents are available for evaluation including the preferred route of administration of oral gavage. Some of the studies listed in Tables 3.11 and 3.12 are of good quality and reliability, as indicated in the tables and by the Klimisch scores (reported where available). Consequently, the standard default assessment factor of 1 should be applied.

Therefore;

- $DNEL_{Oral}$ = $NOAEL_{corrected}$/AF1 x AF2 x AF3 x AF4 x AF5

  = 20/4 x 5 x 1 x 2 x 1

  = **0.5 mg/kg/day**

Dermal

Selection of relevant dose-descriptor: PoD 20 mg/kg/day (as indicated above)

Modification of relevant dose-descriptor: Convert rat oral NOAEL dose-descriptor into dermal rat NOAEL by correcting for differences in absorption between the two routes. As there are no specific absorption data available by these routes of administration, it will be assumed that there is 100% absorption as the worst-case scenario and no modification of the dose-descriptor is required.

Application of assessment factors:

- Interspecies differences – apply a factor for allometric scaling (4 for rat) for oral/rat to dermal/human exposure.

- Intraspecies differences - use Worker default of 5 as this will replicate short term exposure in a light-work scenario (open water swimmer).

- Differences in duration of exposure – PoD taken from a subacute toxicity study (90-days duration) and this daily exposure is considered representative of the frequency of exposure in open water swimming. Therefore, AF is 1.

- Issues related to dose response - PoD based on NOAEL. Hydrogen peroxide is classified as Skin Corr. 1A; H314: Causes severe skin burns and eye damage at concentrations above 70%; as Skin Corr. 1B; H314: Causes severe skin burns and eye damage at concentrations of 50 – 70%; and Skin Irrit. 2; H315: Causes skin irritation at concentrations lower than 50% and above 8%. Therefore, an AF of 3 is used to account for this level of severity of local toxicity.

- Quality of whole database – several oral toxicity studies in rodents are available for evaluation including the preferred route of administration of oral gavage. Some of the studies listed in Tables 3.11 and 3.12 are of good quality and reliability, as indicated in the tables and by the Klimisch scores (reported where available). Consequently, the standard default assessment factor of 1 should be applied.

Therefore;

- $DNEL_{Dermal}$ = $NOAEL_{corrected}$/AF1 x AF2 x AF3 x AF4 x AF5

  = 20/4 x 5 x 1 x 3 x 1

  = **0.33 mg/kg/day**

4 RISK ASSESSMENT

In addition to the guidance on the derivation of exposure values given by ECHA 2008[8] it is also necessary to describe the exact conditions under which the exposures could be expected. To do this, appropriate details of modelling swimming scenarios have been taken from the US EPA Swimmer Exposure Assessment Model[9].

Prior to the determination of the potential risk of open-water swimmers to the three salmon treatments described, azamethiphos, deltamethrin and hydrogen peroxide, it is necessary to model the likely exposure scenario to a wild swimmer during what could be considered a routine period of swimming and "standard" conditions.

Exposure to the treatment substances during a swimming session is demonstrated by the following major routes of absorption into the body:

- Dermal: Absorption of the chemical via the skin will be dependent on the surface area exposed and the Permeability Coefficient (Kp – cm/hr). Assuming that no protection against absorption is offered by the wearing of protective clothing (wet suit) then the dermal route is likely to represent the greatest potential route of exposure during a swimming session;

- Oral: A certain amount of water will inevitably be swallowed during a swimming session, but oral ingestion is considered to be the intermediate mode of exposure and will be dependent on the estimated amount of contaminated water that is swallowed.

Any potential exposure via the inhalation route has been assessed qualitatively from local and acute/repeat-dose toxicity data and appropriate adjustments made to the assessment factors applied to the DNEL calculations.

SWIMODEL characteristics

The US EPA SWIMODEL is designed for use for competition and recreational swimming within the enclosed environment of indoor swimming pools and exposures to known chemicals. However, in the absence of specific models designed for open-water scenarios, SWIMODEL is considered sufficiently reliable to allow the estimation of safe exposure concentrations of fish farm medicines for open water swimmers. The data taken from the SWIMODEL guidance document (US EPA 2003) represent mean data from male and female adults and can be applied to the risk assessment calculations:

- Body weight: 71.8 kg (mean of male and female adult) OR 0.0718 m^3

[8] ECHA Guidance on Information Requirements and Chemical Safety assessment. Chapter R7a: Endpoint Specific guidance. Version 6.0. July 2017
[9] US EPA Swimmer Exposure Assessment Model (SWIMODEL) Version 3.0 (2003)

- Total body surface area: 1.82 m^2 (mean of male and female adult)
- Exposure time: 2 hours (estimated average swimming time per occasion)
- Ingestion rate: 25 mL/hour (representative of light activity)
-

The predicted swim-water concentrations are derived based on a number of worst case assumptions:

- That the water concentration is constant irrespective of environmental conditions e.g. temperature, wind, water flow etc.;
- That the water concentration is constant irrespective of treatment frequency;
- That the swimmer is swimming through a static plume, with no adjustment for distance from farm or distance travelled while swimming;
- No allowance for residue degradation or dilution of the substances in the water;
- 100% absorption by dermal and oral routes of exposure;
- No allowance for metabolism or excretion;
- A standard 71.8 Kg adult human (no modelling for younger adults or children and no sex difference allowance);
- A 2 hour swim with no protection (e.g. wet suit);
- Application of standards based on SWIMODEL data (US EPA 2003).

4.1 Azamethiphos

Oral exposure

The first step is to rearrange the exposure equation from SWIMODEL to:

$$C_w = PDR_{oral} / (ET \times IR)$$

PDR: Potential dose rate via oral exposure (mg/swim)
IR: Ingestion rate (L/hour)
ET: Exposure time (hours/swim)
C_w: Concentration in water (mg/L)

We then convert the oral DNEL for azamethiphos to the potential dose rate via oral exposure (mg/swim):

$$PDR_{oral} = DNEL_{oral} \times \text{body weight} / \text{swim period}$$

$$= (0.00125 \times 71.8 / 24) \times 2$$

$$= 0.007479 \text{ mg/swim}$$

Therefore, the equation can be used to derive the predicted no effect concentration in water that should not be exceeded:

$$C_w = 0.007479 \text{ (mg/swim)} / (2 \text{ (hours)} \times 0.025 \text{ (L/hour)})$$

$$= 0.150 \text{ mg/L}$$

Dermal exposure

The first step is to rearrange the exposure equation to:

$$C_w = PDR_{dermal} / (ET \times SA \times K_p \times (10{,}000 \text{ cm}^2/\text{m}^2) \times (0.001 \text{ L/cm}^3))$$

PDR: Potential dose rate via dermal exposure (mg/swim)
ET: Exposure time (hours/swim)
SA: Skin surface area (m^2)
C_w: Concentration in water (mg/L)
K_p: Chemical specific permeability coefficient (cm/hour)

We then convert the dermal DNEL for azamethiphos to the potential dose rate via dermal exposure (mg/swim):

$$PDR_{dermal} = DNEL_{dermal} \times \text{body weight / swim period}$$

$$= (0.0025 \times 71.8 / 24) \times 2$$

$$= 0.01496 \text{ mg/swim}$$

Therefore, the equation can be used to derive the predicted no effect concentration in water that should not be exceeded:

$$C_w = 0.01496 \text{ (mg/swim)} / (2 \text{ (hours)} \times 1.82 \text{ (m}^2\text{)} \times 1E\text{-}03 \text{ (cm/hour)} \times 10000 \text{ (cm}^2/\text{m}^2\text{)} \times 0.001 \text{ (L/cm}^3\text{))}$$

$$= 0.411 \text{ mg/L}$$

4.2 Deltamethrin

Oral exposure

The first step is to rearrange the exposure equation from SWIMODEL to:

$$C_w = PDR_{oral} / (ET \times IR)$$

PDR: Potential dose rate via oral exposure (mg/swim)
IR: Ingestion rate (L/hour)

ET: Exposure time (hours/swim)
C_w: Concentration in water (mg/L)

We then convert the oral DNEL for deltamethrin to the potential dose rate via oral exposure (mg/swim):

$$PDR_{oral} = DNEL_{oral} \text{ x body weight / swim period}$$

$$= (0.025 \text{ x } 71.8 / 24) \text{ x } 2$$

$$= 0.1496 \text{ mg/swim}$$

Therefore, the equation can be used to derive the predicted no effect concentration in water that should not be exceeded:

$$C_w = 0.1496 \text{ (mg/swim)} / (2 \text{ (hours)} \text{ x } 0.025 \text{ (L/hour)})$$

$$= 2.992 \text{ mg/L}$$

Dermal exposure

The first step is to rearrange the exposure equation to:

$$C_w = PDR_{dermal} / (ET \text{ x } SA \text{ x } K_p \text{ x } (10{,}000 \text{ cm}^2/\text{m}^2) \text{ x } (0.001 \text{ L/cm}^3))$$

PDR: Potential dose rate via dermal exposure (mg/swim)
ET: Exposure time (hours/swim)
SA: Skin surface area (m^2)
C_w: Concentration in water (mg/L)
K_p: Chemical specific permeability coefficient (cm/hour)

We then convert the dermal DNEL for deltamethrin to the potential dose rate via dermal exposure (mg/swim):

$$PDR_{dermal} = DNEL_{dermal} \text{ x body weight / swim period}$$

$$= (0.05 \text{ x } 71.8 / 24) \text{ x } 2$$

$$= 0.2992 \text{ mg/swim}$$

Therefore, the equation can be used to derive the predicted no effect concentration in water that should not be exceeded:

$$C_w = 0.2992 \text{ (mg/swim)} / (2 \text{ (hours)} \text{ x } 1.82 \text{ (m}^2\text{)} \text{ x } 1E\text{-}03 \text{ (cm/hour)} \text{ x } 10000 \text{ (cm}^2/\text{m}^2\text{)} \text{ x } 0.001 \text{ (L/cm}^3\text{))}$$

$$= 8.22 \text{ mg/L}$$

4.3 Hydrogen peroxide

Oral exposure

The first step is to rearrange the exposure equation from SWIMODEL to:

$$C_w = PDR_{oral} / (ET \times IR)$$

PDR: Potential dose rate via oral exposure (mg/swim)
IR: Ingestion rate (L/hour)
ET: Exposure time (hours/swim)
C_w: Concentration in water (mg/L)

We then convert the oral DNEL for hydrogen peroxide to the potential dose rate via oral exposure (mg/swim):

$$PDR_{oral} = DNEL_{oral} \times \text{body weight / swim period}$$

$$= (0.5 \times 71.8 / 24) \times 2$$

$$= 2.992 \text{ mg/swim}$$

Therefore, the equation can be used to derive the predicted no effect concentration in water that should not be exceeded:

$$C_w = 2.992 \text{ (mg/swim)} / (2 \text{ (hours)} \times 0.025 \text{ (L/hour)})$$

$$= 59.84 \text{ mg/L}$$

Dermal exposure

The first step is to rearrange the exposure equation to:

$$C_w = PDR_{dermal} / (ET \times SA \times K_p \times (10{,}000 \text{ cm}^2/\text{m}^2) \times (0.001 \text{ L/cm}^3))$$

PDR: Potential dose rate via dermal exposure (mg/swim)
ET: Exposure time (hours/swim)
SA: Skin surface area (m^2)
C_w: Concentration in water (mg/L)
K_p: Chemical specific permeability coefficient (cm/hour)

We then convert the dermal DNEL for hydrogen peroxide to the potential dose rate via dermal exposure (mg/swim):

$$PDR_{dermal} = DNEL_{dermal} \times \text{body weight / swim period}$$

$$= (0.33 \times 71.8 / 24) \times 2$$

$$= 1.9745 \text{ mg/swim}$$

Therefore, the equation can be used to derive the predicted no effect concentration in water that should not be exceeded:

C_w = 1.9745 (mg/swim) / (2 (hours) x 1.82 (m^2) x 1E-03 (cm/hour) x 10000 (cm^2/m^2) x 0.001 (L/cm^3))

= 54.24 mg/L

4.4 Risk Characterisation

The risk characterisation ratios (RCRs) have been calculated for each of the substances, based on a comparison of the lowest concentrations predicted to present no hazard to swimmers (on a worst-case basis) with the concentration of the substances in the treatment baths. An RCR of lower than 1, indicates no risk, and an RCR above 1 indicates a potential risk.

RCR = Concentration in treatment bath / Predicted safe swim water concentration

Azamethiphos

For azamethiphos, the lowest predicted safe swim-water concentration is via the oral route of exposure. The value was determined to be 0.150 mg/L, and the concentration of azamethiphos in the treatment bath was given as 0.120 mg/L. Therefore:

RCR = 0.120 / 0.150

= 0.80

Therefore, this indicates that the levels of azamethiphos in the treatment bath can be considered safe.

Deltamethrin

For deltamethrin, the lowest predicted safe swim-water concentration is via the oral route of exposure. The value was determined to be 2.99 mg/L, and the concentration of deltamethrin in the treatment bath was given as 0.002 mg/L. Therefore:

RCR = 0.002 / 2.99

= 0.00067

Therefore, this indicates that the levels of deltamethrin in the treatment bath can be considered safe.

Hydrogen peroxide

For hydrogen peroxide, the lowest predicted safe swim-water concentration is via the dermal route of exposure. The value was determined to be 54.24 mg/L, and the concentration of hydrogen peroxide in the treatment bath was given as 1500 mg/L. Therefore:

RCR = 1500 / 54.24

= 27.7

Therefore, this indicates that the levels of hydrogen peroxide in the treatment bath cannot be considered safe. Characterisation of dilution and dispersion factors are likely to be required to be taken into account to demonstrate that discharges of hydrogen peroxide are safe for open-water swimmers[10].

[10] An assessment of such dilution and dispersion characteristics for hydrogen peroxide (undertaken by Salmon Scotland) is given in Appendix 1.

5 CONCLUSIONS

In Table 5.1, the determined safe limits of the three chemicals are shown in relation to their route of exposure. The overall safe limit is considered to be the lowest of the limits derived for the two major routes of exposure. The table also demonstrates the concentrations of the substances used in the treatment baths, and the calculated risk characterisation ratios (RCRs) for the substances.

Table 5.1 Risk assessment summary for the three substances

Data	Information	Azamethiphos	Deltamethrin	Hydrogen peroxide
SWIMODEL water concentrations (mg/L)	Oral	0.150	2.99	59.84
	Dermal	0.411	8.22	54.24
	Lowest	0.150	2.99	54.24
Maximum concentration used to treat fish (mg/L)		0.12	0.002	1500
Risk characterisation ratio (RCR)		0.8	0.0007	27.7

For Azamethiphos, the oral and dermal predicted no-effect limits are similar, with the oral limit being lower and taken for the risk assessment. The predicted no-effect limit was determined to be 0.150 mg/L via the oral route, and the risk characterisation ratio was calculated to be 0.8. As this value is lower than 1, this indicates that the levels of azamethiphos in the treatment bath can be considered safe.

For deltamethrin, the oral and dermal predicted no-effect limits are similar, with the oral limit being lower and taken for the risk assessment. The predicted no-effect limit was determined to be 2.99 mg/L via the oral route, and the risk characterisation ratio was calculated to be 0.0067. As this value is lower than 1, this indicates that the levels of azamethiphos in the treatment bath can be considered safe.

For hydrogen peroxide, the oral and dermal predicted no-effect limits are similar, with the dermal limit being lower and taken for the risk assessment. The predicted no-effect limit was determined to be 54.24 mg/L via the dermal route, and the risk characterisation ratio was calculated to be 27.7. As this value is above 1, this indicates that of hydrogen peroxide in the treatment bath present a risk. However, the process followed in this document followed a "worst-case scenario" basis, therefore, the levels of hydrogen peroxide in the swimming area may be considerably lower than in the treatment bath[11].

It is important to note the details of the open-water swimmer model to which these data relate (Section 4), since other standard values are available depending on the age and sex of the swimmer as well as the level of work being undertaken by the swimmer. There may be other

[11] An assessment of such dilution and dispersion characteristics for hydrogen peroxide (undertaken by Salmon Scotland) is given in Appendix 1.

circumstances that could affect the exposures to the swimmers that have not been taken into consideration such as air and water temperatures, but these were outside the scope of this assessment.

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Appendix 1

Hydrogen Peroxide dispersion model

Details generated by Salmon Scotland

Summary

In order to place the results in the main report into context, the dispersion of hydrogen peroxide from a pen following a tarpaulin treatment was assessed using a modified version of "BathAuto" model. This model has been used by SEPA for regulatory purposes (to calculate short-term dispersion of bath treatments from marine pen fish farms) for around 25 years. The spatial and temporal scale of impact relative to the No Effects Level (NEL = 59.84 mg l^{-1}) was computed. The model allows for a generic risk assessment approach for a range of initial treatment quantities and environmental conditions (mean current speeds) typical of marine fish farms.

Methods

The model is based on the approximation of the discharge patch as an elliptic cylinder, the volume of which is calculated at the time of interest and compared to a specified concentration (Environmental Quality Standard). In a regulatory context, predictions for Azamethiphos & Deltamethrin are used to determine the maximum discharge quantities that are likely to satisfy these standards. Hydrogen peroxide is considered of lower environmental concern and is not controlled through specific discharge limits, rather it is controlled through general permit conditions. In the context of this modelling exercise the calculations were modified to estimate the time taken for concentrations to fall below the NEL.

The area of the patch is calculated from the mean current speed (patch length) and a fixed dispersion coefficient (patch width). The area of the patch is therefore a function of time, and was calculated at 1-minute intervals. The moment at which the patch exceeds the pen area was considered to be equivalent to the time of tarpaulin release; all statistics were calculated based on dispersion starting from this moment in time.

Several key statistics were calculated: i) time taken from tarpaulin release for peak concentration to drop below the NEL given in the main report; ii) distance travelled by the patch over this time; iii) peak and mean (over area) patch concentrations at 2 hrs post release; iv) average of patch concentration over first 2 hrs post release (average of both peak and mean concentrations over time). The latter allows comparison against the NELs given in the report, which assume a 2 hr exposure. To ensure that results were suitably precautionary, the model was run based on two of the largest pen sizes (with correspondingly large treatment mass) in use in Scotland at present: 120 and 160 m circumference, although larger pen sizes tend to be used primarily in faster current regimes, further from shore (many sites use 80-100 m pens).

Results

For both pen sizes considered, peak concentration within the patch is predicted to be below the NEL in around 30-60 minutes for most scenarios, with a maximum time of 100 minutes. The distance travelled by the patch centre during this time ranged from 164-378 m. Peak and average concentrations within the patch were correspondingly well below the NEL at the 2 hr point.

The 2 hr average of peak patch concentration was over the NEL (1.2-2.4 x for 120 m pens, and higher (1.5-3.2 x) for 160 m pens due to greater treatment mass and volume). The 2 hr average of mean patch concentration was below or very close to the NEL for 120 pens in all but the slowest current scenario (where it was 1.4 x NEL over 2 hrs). For 160 m pens, 2 hr average of mean values was only below the NEL at the fastest current speed (worst case 1.9 x at slowest current speed).

Conclusions and context

It is clear from the results that while pen concentrations of H_2O_2 are much higher than the reported NELs, dispersion in an open-water environment is expected to reduce concentration below the NELs quickly, in as little as 30 minutes and generally within a distance of 2-300 m from the treated pen centre point. Moreover, in many cases (and particularly the smaller pen scenarios, which are more realistic for the types of environment which swimmers will use), the 2 hr average of the mean patch concentration is below the reported NEL.

Even in the worst-case scenario (an unrealistic combination of very large pen and very slow current speed), the average of the peak concentration over 2 hrs is 3.2 x NEL. To experience such concentrations, a swimmer would have to be at the pen edge at the moment the tarpaulin was dropped, and swim following the central peak of the patch (most likely parallel to the coastline) for a 2 hr period. Very few (if any) swimmers in Scottish coastal waters will swim for 2 hrs, with a more common swim duration being 30-45 minutes. Allowing for the time taken to swim to a farm (typically over 100 m from the shore), and the need to time the swim perfectly with medicine release and movement, exposure at this level would appear to be exceedingly unlikely. If swimmers follow guidance of remaining outside pen grid marker buoys, risk of exposure is reduced even further.

It should also be borne in mind that most swimmers in Scottish coastal waters for the durations modelled here, will be wearing a wetsuit[1,2], offering added protection.

Table 1 Output from the modified BathAuto short-term model considering 120m and 160m circumference pens assuming a 4m treatment depth for mean current speeds ranging from 0.04 to 0.16m s^{-1}. Times are given from the moment of tarpaulin release. The NEL calculated for Hydrogen Peroxide in the main report was 59.84 mg l^{-1}.

120m circumference pens	**Mean current speed (m s^{-1})**				
	0.04	**0.07**	**0.10**	**0.13**	**0.16**
Time until peak concentration in the patch <NEL (min)	68.3	47.0	37.1	31.1	27.3
Potential distance travelled by the patch when peak concentration <NEL (m)	163.94	197.36	222.85	242.25	261.77
Peak concentration in the patch after 2 hrs (mg l^{-1})	28.93	17.31	12.41	9.66	7.94
Mean concentration in the patch after 2 hrs (mg l^{-1})	17.36	10.39	7.44	5.80	4.77
Average of peak concentration over first 2 hrs (mg l^{-1})	143.26	106.40	88.63	75.75	69.66
Average of mean patch concentration over first 2 hrs (mg l^{-1})	85.95	63.84	53.18	45.45	41.80
160m circumference pens	**Mean current speed (m s^{-1})**				

	0.04	**0.07**	**0.10**	**0.13**	**0.16**
Time until peak concentration in the patch <NEL (min)	100.3	69.2	54.8	45.4	39.4
Potential distance travelled by the patch when peak concentration <NEL (m)	240.78	290.50	328.67	353.99	377.79
Peak concentration in the patch after 2 hrs (mg l^{-1})	48.11	29.39	21.29	16.57	13.62
Mean concentration in the patch after 2 hrs (mg l^{-1})	28.87	17.63	12.78	9.94	8.17
Average of peak concentration over first 2 hrs (mg l^{-1})	191.75	145.53	122.95	101.85	89.82
Average of mean patch concentration over first 2 hrs (mg l^{-1})	115.05	87.32	73.77	61.11	53.89