EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

19 April 2021
EMA/CVMP/223046/2021
Veterinary Medicines Division

Committee for Medicinal Products for Veterinary Use

European public MRL assessment report (EPMAR)

Imidacloprid (fin fish)

On 15 April 2021 the European Commission adopted a Regulation[1] establishing maximum residue limits for imidacloprid in fin fish, valid throughout the European Union. These maximum residue limits were based on the favourable opinion and the assessment report adopted by the Committee for Medicinal Products for Veterinary Use.

Imidacloprid is intended for use in Atlantic salmon (*Salmo salar*) for the control of sea-lice (*Lepeophtheirus salmonis*).

Benchmark Animal Health Norway AS submitted to the European Medicines Agency an application for the establishment of maximum residue limits on 23 April 2019.

Based on the original and complementary data in the dossier, the Committee for Medicinal Products for Veterinary Use recommended on 9 September 2020 the establishment of maximum residue limits for imidacloprid in fin fish.

Subsequently the Commission recommended on 26 February 2021 that maximum residue limits in fin fish are established. This recommendation was confirmed on 20 March 2021 by the Standing Committee on Veterinary Medicinal Products and adopted by the European Commission on 15 April 2021.

[1] Commission Implementing Regulation (EU) No 2021/621, O.J. L 131, of 16 April 2021

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Summary of the scientific discussion for the establishment of MRLs

Substance name:	Imidacloprid
Therapeutic class:	NO ENTRY
Procedure number:	EMEA/V/MRL/004481/FULL/0002
Applicant:	Benchmark Animal Health Norway AS
Target species requested:	*Salmonidae*
Intended therapeutic indication:	Ectoparasiticide
Route(s) of administration:	Topical use

1. Introduction

Imidacloprid is a chloronicotinyl compound belonging to the neonicotinoid group, and it is used as a systemic insecticide. Its insecticide effects are exerted by the substance binding irreversibly to specific insect nicotinic acetylcholine receptors, interfering with the transmission of nerve impulses in insects.

An application was submitted by Benchmark Animal Health Norway AS to the European Medicines Agency for the establishment of maximum residue limits for imidacloprid, to be used in a product for the control of sea-lice (*Lepeophtheirus salmonis*) in Atlantic salmon (*Salmo salar*). Imidacloprid would be used topically, by being solubilised in water at a concentration of 20 mg/l and animals would be immersed in the solution for 60 minutes.

2. Scientific risk assessment

2.1. Safety assessment

The scientific risk assessment relies partly on the assessment of the Joint WHO/FAO Meeting on Pesticides (JMPR, 2002)[2]. However, the data evaluated by the JMPR were not available. Therefore, new pivotal (GLP) studies were submitted to allow an independent assessment by the CVMP. The parts of the assessment relying on the JMPR assessment are indicated as such, and a brief summary of the studies is provided.

2.1.1. Overview of pharmacological properties

Pharmacodynamic properties including mode of action

Neonicotinoid compounds are structurally related to nicotine, and like nicotine they bind to the nicotinic acetylcholine receptors (nAChRs) present on neural cells. By blocking nicotinic acetylcholine receptors, imidacloprid prevents acetylcholine from transmitting impulses between nerves, resulting in the insect's paralysis and eventual death. The selective effects of imidacloprid to insects, but not to mammals, are attributed to its binding affinity at the nicotinic receptor, and in turn, related to subtype, function and neuronal region. Hence, neonicotinoids have low mammalian toxicity but are highly toxic to insects. Specifically, in mice, imidacloprid was found to bind to the α4β2 nicotinic acetylcholine receptor with an IC_{50} of 2600 nM, compared to 7.0 nM for nicotine itself, and 8.2 nM for the desnitro

[2] JMPR (Joint FAO/WHO Meeting on Pesticide Residues) (2002). Imidacloprid. Pesticides in Food – 2001, Evaluations 2001, Part II – Toxicological, International Programme on Chemical Safety, World Health Organisation, Geneva, 2002, 79-100

derivative.

No specific pharmacological studies are available. The neurological effects related to the mechanism of action are expected to be observable in the toxicology studies, including studies on neuro-developmental toxicity.

Pharmacokinetic properties (mainly in laboratory animals)

The pharmacokinetic properties of imidacloprid in rats/mammals have been summarised by JMPR. The substance is well absorbed, and widely distributed to body tissues, with low levels in adipose tissues and the nervous system. Imidacloprid and/or metabolites undergo extensive entero-hepatic cycling, but it is still rapidly excreted, mainly via urine. The main metabolites in rats are 6-chloronicotinic acid and 4- or 5-hydroximidacloprid.

The finding of the nitroso metabolite in rat urine after chronic high exposure (as reported by JMPR) may be of minor relevance to the safety evaluation of imidacloprid residues, as there is no concern for genotoxicity.

2.1.2. Calculation of pharmacological ADI, if relevant

The pharmacological effects of imidacloprid are related to the binding to the nicotinic receptor of neurons, preventing signal transmissions. Due to the different make-up of this receptor and its subunits in insects and mammals, the substance has a different binding affinity and therefore is highly neurotoxic to insects, and much less neurotoxic to mammals. The neurotoxic effects of imidacloprid will be covered by the toxicity studies, and therefore the establishment of a separate pharmacological ADI for imidacloprid is not considered necessary.

2.1.3. Overview of toxicology

In addition to the information on the toxicity of imidacloprid coming from the JMPR evaluation, the following new studies were provided by the applicant:

- An acute oral toxicity study in the rat, using the up and down procedure;
- A 12-month dietary toxicity study in the rat with standard arena observations and a functional observation battery;
- An extended one generation reproductive study in the rat with cohorts for the investigation of neurotoxicity and immunotoxicity;
- A pre-natal developmental toxicity study in the rat;
- A bacterial reverse mutation study;
- An *in vitro* micronucleus study in human lymphocytes;
- An oral micronucleus study in the rat.

The summaries and conclusions of the new studies are reported in the relevant sections below.

Single-dose toxicity

In the new acute oral toxicity study in the rat, no clinical signs occurred at 130 mg/kg bw. At 410 mg/kg bw, twitching, decreased activity, noisy breathing and hunched posture were reported in one animal. No clinical signs were seen in the other two animals given this dose. At 1300 mg/kg bw, clinical signs included decreased activity, twitching, laboured or noisy breathing, ptosis and prone posture in 3 animals, which died later. The remaining animals given this dose showed no clinical signs. The single

animal given 2000 mg/kg bw developed twitching and decreased activity within 1 hour of dosing and died 1 hour after dosing, after developing clonic convulsions.

In the JMPR summary report, it is stated that following a single oral administration, imidacloprid was moderately toxic to rats (LD_{50} 380-650 mg/kg bw), and mice (LD_{50} 130-170 mg/kg bw). Behavioural and respiratory signs, disturbances of motility, narrowed palpebral fissures, transient trembling and spasms were seen in rats and mice treated orally at doses of 200 mg/kg bw and above and 71 mg/kg bw and above, respectively. The clinical signs were reversed within 6 days.

Repeated dose toxicity

No new studies on repeated-dose 90-day oral toxicity were submitted with this application. For reference, 90-days studies in rats and in other species available in the JMPR report are briefly reviewed below, as are relevant studies from the scientific literature.

Groups of 10 male and 10 female Wistar rats were given diets containing imidacloprid at concentrations of 0, 120, 600 or 3000 ppm imidacloprid, for up to 98 days in a non-GLP compliant study. These concentrations were equivalent to 0, 11, 57 and 410 mg/kg bw/day for males and 0, 15, 78 and 510 mg/kg bw/day for females. Food intake was increased in rats given 3000 ppm, while body weights were reduced at 600 and 3000 ppm. There was a significant increase in alkaline phosphatase activity at 3000 ppm, while males showed decreased cholesterol. Degenerative changes occurred in the testes of 5 out of 10 males at 3000 ppm, and multifocal group cell necrosis occurred in the liver of one male at 3000 ppm. The NOAEL in this study, as established by the JMPR, was 120 ppm or 11 mg/kg bw/day.

Groups of 10 male and 10 female Wistar rats were given diets containing 0, 150, 600 or 2400 ppm imidacloprid for up to 96 days in a GLP-compliant study. Satellite groups of 10 male and 10 female rats were given diets containing 0 or 2400 ppm imidacloprid for the same period, but continued on control diets after this time and for a period of four weeks, for investigating the reversibility of any adverse effects. In animals given 2400 ppm imidacloprid, feed intake was increased during treatment, as well as during the recovery period in the satellite group. Reduced body weight gains occurred in males at 600 ppm and in females at 2400 ppm. Slightly longer prothrombin times, and slightly depressed thrombocyte counts were found at 2400 ppm imidacloprid, and these effects were only partially reversible in the recovery group animals. Elevated alkaline phosphatase and alanine aminotransferase activities with lower protein, albumin, cholesterol and triglycerides occurred in animals of both sexes at 2400 ppm. Depressed protein concentrations occurred in males at 600 ppm. At 2400 ppm imidacloprid, males had increased incidences of hepatocellular necrosis, round-cell (lymphocyte) infiltration, swollen nuclei and cytoplasmic lesions, but these effects were reversed in the 4-week recovery period. The NOEL in this study, as established by the JMPR, was 150 ppm or 14 mg/kg bw/day.

In a published study, groups of 10 female Wistar rats were given oral doses of 0, 5, 10 or 20 mg/kg bw/day imidacloprid in corn oil by gavage, for 90 days. There were no adverse effects at 5 or 10 mg/kg bw/day. However, after 90 days at 20 mg/kg bw/day, food consumption and body weights were reduced. At this dose, there was a decrease in spontaneous locomotor activity, lower brain and plasma acetylcholinesterase, and degenerative effects in the cerebellum, liver and kidneys. There were also effects on clinical chemistry. The NOAEL in this study was 10 mg/kg bw/day (Bhardwaj *et al.*, 2010)[3].

In another published study by the same group of investigators, groups of 5 female Wistar rats were given doses of 0, 5, 10 or 20 mg/kg bw/day imidacloprid for 90 days. Activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were determined in selected

[3] Bhardwaj, S., Srivastava, M. K., Kapoor, U., & Srivastava, L. P. (2010). A 90 days oral toxicity of imidacloprid in female rats: morphological, biochemical and histopathological evaluations. *Food and chemical toxicology*, *48*(5), 1185-1190

tissues, and glutathione (GSH) content and lipid peroxidation measured. Tissues selected were liver, kidney and brain. Doses of 5 or 10 mg/kg bw/day had no effects on any of the parameters investigated. However, at 20 mg/kg bw/day lipid peroxidation was significantly increased in liver and kidney while SOD, CAT and GPx were increased in liver and brain, while GSH was increased in liver. The effects noted may be indicative of oxidative stress. The NOAEL in this study was 10 mg/kg bw/day (Kapoor *et al.*, 2010)[4].

In what appears to be an extension of the above study, groups of 10 female Wistar rats were given gavage doses of imidacloprid at 0, 5, 10 or 20 mg/kg bw/day in corn oil for 90 days to specifically examine its effects on ovarian morphology, hormones and antioxidant enzymes. There were no signs of overt toxicity at 5 or 10 mg/kg bw/day but animals given 20 mg/kg bw/day showed a significant reduction in weight gain, piloerection, dyspnoea, salivation and diarrhoea. Animals given the highest dose showed cytoplasmic clumping and abundant lipofuscin in the granulosa cells of the ovarian follicles. There were no similar abnormalities in the other dose groups. Serum follicle-stimulating hormone (FSH) was increased while Luteinizing hormone (LH) and progesterone were decreased in high dose animals. At 5 or 10 mg/kg bw/day, there were no effects in ovarian lipid peroxidation, but at 20 mg/kg bw/day there was an increase in ovarian lipid peroxidation, and decreases in GSH content, SOD, CAT and GPx. The results suggest an increase in oxidative stress in the ovaries of females exposed to the highest dose. The NOAEL in this study was 10 mg/kg bw/day (Kapoor *et al*., 2011)[5].

In a non-GLP compliant study with imidacloprid, groups of 10 male and 10 female B6C3F1 mice were given diets containing 0, 120, 600 or 3000 ppm imidacloprid for up to 107 days, resulting in oral doses of 0, 17, 86 and 430 mg/kg bw/day. At the highest dietary concentration, 7 out of 10 males and 7 out of 10 females died, and several mice showed poor condition and rough coats. Body weight gain was reduced and food consumption was increased in males at 600 ppm and in males and females at 3000 ppm. At the highest dietary level, blood urea and cholesterol were decreased in males while alanine aminotransferase and glucose was lower in females. Alkaline phosphatase activity was increased in males and females at 3000 ppm and in females and at 120 and 600 ppm. There were effects on the weights of the liver, heart, spleen, kidneys, testes and adrenals at 3000 ppm. The NOAEL in this study, as established by the JMPR, was 120 ppm equivalent to 17 mg/kg bw/day.

In a GLP study, groups of 4 male and 4 female beagles were given diets containing imidacloprid at concentrations of 0, 200, 600 or 1800/1200 ppm for 13 weeks. At the highest concentration, the level of dietary imidacloprid was reduced from 1800 ppm to 1200 ppm after 4 weeks due to low food intake. After being transferred to the diet containing 1200 ppm imidacloprid, the body weight gain began to return to normal. Food consumption was also reduced at 600 ppm. At the higher concentrations, animals showed an emaciated state and transient trembling. The NOAEL in this study, as established by the JMPR, was 200 ppm or 7.5 mg/kg bw/day.

In the pivotal, GLP-compliant 1 year repeated dose oral toxicity in rats, provided by the applicant, groups of 20 male and 20 female Crl:WI(Han) rats received imidacloprid in the diet at concentrations of 0, 100, 300, and 1000 ppm, equivalent to 0/0, 5.6/6.7, 16.3/19.5, and 55.9/63.7 mg/kg bw/day for males and females, respectively. The study included standard arena observations, and a functional observation battery. There were no effects apart from a dose-related decreased body weight gain at all dose levels, accompanied by a decreased feed intake, although the change was not statistically significant and less than 10% at the lowest dose. Since the feed intake was also reduced in three 90-days studies using oral gavage (see above), and therefore may not be (completely) attributable to

[4] Kapoor, U., Srivastava, M. K., Bhardwaj, S., & Srivastava, L. P. (2010). Effect of imidacloprid on antioxidant enzymes and lipid peroxidation in female rats to derive its No Observed Effect Level (NOEL). The Journal of toxicological sciences, 35(4), 577-581.
[5] Kapoor, U., Srivastava, M. K., & Srivastava, L. P. (2011). Toxicological impact of technical imidacloprid on ovarian morphology, hormones and antioxidant enzymes in female rats. Food and chemical toxicology, 49(12), 3086-3089.

palatability issues, the CVMP derived a NOAEL of 5.6 mg/kg bw/day, based on dose-related changes in bodyweights at higher doses.

A further 52-week oral repeated dose study in dogs was reported by the JMPR. In this GLP study, groups of 4 male and 4 female beagles were given diets containing imidacloprid at concentrations of 0, 200, 500 or 1250/2500 ppm for 52 weeks. After 17 weeks, the 1250 ppm diet was increased to 2500 ppm. Slight, temporary reductions in food intake occurred at 1250 ppm and at the increased concentration of 2500 ppm. At the highest dietary concentration, an increase in plasma cholesterol was noted at 13 and 26 weeks in females, and an increase in hepatic cytochrome P450 in males and females at 52 weeks. The NOAEL in this study, as established by the JMPR, was 500 ppm or 15 mg/kg bw/day.

Reproductive toxicity, including developmental toxicity

In a recent well-conducted, GLP-compliant extended one generation reproductive toxicity study (EOGRTS) in the rat, performed in accordance with OECD guideline 443, groups of 24 male and 24 female rats were given imidacloprid at dietary concentrations of 0, 100, 300 or 1000 ppm. Males were treated for 70 days, 2 weeks prior to pairing and mating, and throughout pairing and mating. Females were treated for up to 72 days, 2 weeks prior to pairing, during pairing and gestation and up to lactation day 21 and weaning of the F1 generation. F1 pups were divided into 5 cohorts for the investigation of different endpoints (systemic toxicity and reproduction, reproductive toxicity, neurotoxicity, neuropathology, and immunotoxicity), and these were given dietary imidacloprid at 0, 100, 300 or 1000 ppm for varying periods depending on the study endpoint. Statistically significant reductions in body weight were seen in F_0 animals at 300 and 1000 ppm. In this study, there was no evidence of reproductive or developmental toxicity, nor evidence of neurobehavioral effects, developmental neurotoxicity, or neuropathological effects in the neonates. In the immunotoxicity cohort, a dose-dependent reduction of T-cell dependent antibody response was apparent in females at all doses levels, which did not reach statistical significance due to high variances in the results. Moreover, there was a slight reduction of group average mean cell counts for total T-lymphocytes, helper T-lymphocytes and cytotoxic T-lymphocytes in males. In addition, moderate imidacloprid related changes on organ weights, macroscopical and microscopical effects were observed in thymus, spleen and lymph nodes at 1000 ppm and red to dark pigmentation in thymus in females from 300 ppm on. In addition, the study did reveal a number of systemic effects. The centrilobular hepatocellular eosinophilia/hypertrophy in all males and in mid and high dosed females was considered as a non-adverse adaptive response. An increase in the incidence and severity of physiologic hyperplasia of the mammary glands in females was observed at all dose groups (the LOAEL for the latter effect was 10.4 mg/kg bw/day, the lowest dose tested in females). At post-natal day 22, T4 levels in pups showed a dose-related increase at all imidacloprid dose levels, reaching statistical significance at 300 ppm in males and at 1000 ppm in males and females. Based on the changes in bodyweights, the CVMP derived a NOAEL for systemic toxicity of 100 ppm (equivalent to 5.25 mg/kg bw/day based on F_0 male reduced body weight), and considered that this NOAEL would also be sufficiently protective for the effects on mammary glands in females observed at doses of 10.4 mg/kg bw/day and above.

In a 2-generation reproductive toxicity study reported by JMPR, no effects on reproduction were noted up to the highest dose level of 47 mg/kg bw/day.

In a well-conducted, GLP-compliant developmental toxicity study in the rat, groups of 22 sexually mature, timed-mated, female Crl:WI(Han) rats were given imidacloprid, once daily by gavage, at doses of 0, 5, 15 or 50 mg/kg bw/day on days 6-19 of gestation. The only effect noted was a reduced feed intake and reduced bodyweight at the highest dose. The NOAEL for maternal toxicity was 15 mg/kg bw/day, and for foetal toxicity was 50 mg/kg bw/day, the highest dose tested. Imidacloprid was not

teratogenic in this study.

In a GLP-compliant study reported by the JMPR, imidacloprid was given to groups of 25 mated female Wistar rats from days 6 to 15 of gestation at doses of 0, 10, 30 or 100 mg/kg bw/day by gavage. Dose-related reductions in food consumption occurred accompanied by reductions in body weight gains, at 30 and 100 mg/kg bw/day. Skeletal examination showed a slightly increased incidence of wavy ribs at 100 mg/kg bw/day. The JMPR established a NOAEL for maternal effects of 10 mg/kg bw/day and for development effects of 30 mg/kg bw/day.

In a published developmental immunotoxicity study in Wistar rats given doses of 0, 10, 30 or 90 mg/kg bw/day from days 6 to 20 of gestation, maternotoxicity and foetotoxicity was noted at 30 and 90 mg/kg bw/day. There were no soft tissue malformations but there was a low incidence of wavy or missing ribs, and delayed ossification at 30 and 90 mg/kg bw/day, which are generally not considered as relevant adverse effects. Furthermore, effects on immunoglobulin concentrations, haemagglutination and delayed type hypersensitivity at dosages of 30 and 90 mg/kg bw/day were described. Effects were minimal at 10 mg/kg bw/day. The LOAEL in this study was 10 mg/kg bw/day (Gawade *et al.,* 2013)[6].

In a GLP-compliant study reported by JMPR, imidacloprid was administered by gavage to groups of 16 female chinchilla (Kfm:CHIN) rabbits at doses of 0, 8, 24 or 72 mg/kg bw/day on days 6 to 18 of gestation. Decreased feed intakes and reductions in body weight gain were noted at doses of 24 mg/kg bw/day and above. Two females given the highest dose died on days 18 and 19 of gestation, respectively. A further female at this dose aborted on day 26, and two females showed total resorption at terminal necropsy. High dose females had slightly post-implantation losses when only animals with live foetuses at termination were considered. This increased to a high loss (32.5%) when females with total resorption or abortion were taken into account. The body weights of foetuses were reduced, and the incidences of foetuses with retarded ossification was increased at 72 mg/kg bw. In this study, the NOAEL for maternal toxicity, as established by the JMPR, was 8 mg/kg bw/day, and that for developmental effects was 24 mg/kg bw/day.

Overall, it can be concluded that imidacloprid is not teratogenic.

Genotoxicity

Two new *in vitro* genotoxicity studies were provided: a test for reverse mutations in bacteria (Ames test) and a test for micronucleus induction. Both studies were conducted under GLP and in accordance with relevant OECD guidelines.

The Ames test was clearly negative at all concentrations and in the presence and absence of the rat S9 metabolic activation system.

In the *in vitro* micronucleus test, negative results were obtained following a 3 hours incubation with concentrations up to 2000 µg/ml with and without metabolic activation. However, when the incubation time was increased to 24 hours and tested without metabolic activation, a statistically significant and dose-dependent increase in the number cells with micronuclei was observed at the two highest concentrations (800 and 1300 µg/ml).

In a newly conducted oral *in vivo* micronucleus study in rats there was no evidence of clastogenicity or aneugenicity following oral (gavage) administration of imidacloprid up to the maximum tolerated dose of 200 mg/kg/day in male rats. Imidacloprid is therefore considered negative in this rat peripheral blood micronucleus test.

[6] Gawade, L., Dadarkar, S. S., Husain, R., & Gatne, M. (2013). A detailed study of developmental immunotoxicity of imidacloprid in Wistar rats. Food and chemical toxicology, 51, 61-70.

On the basis of the studies available to the JMPR in 2002, the JMPR concluded that because "all tests for chromosomal damage *in vivo* (…) were negative, the significance of the clastogenic effect of imidacloprid in human lymphocyte cultures is questionable".

It should be noted that after the JMPR publication, several in vitro and in vivo genotoxicity studies were published, covering positive clastogenic effects and gene mutations in mammalian cells. A number of the published studies were discarded for the assessment of imidacloprid, because they were done using a commercial formulation with no information on the exact composition of the product, hence without any information on the potential effects that the other components in the formulations might have had on the results obtained. Some other published studies were discarded due to experimental deficiencies.

From the remaining reliable *in vitro* studies, it can be concluded that imidacloprid can induce chromosomal aberrations in mammalian cells and can induce gene mutations in human derived TK cells.

I*n vivo* imidacloprid does not induce chromosomal aberrations. However, it has to be noted that the potential of imidacloprid to induce gene mutations was not tested *in vivo*. In light of the negative carcinogenicity results in two species, the absence of an *in vivo* study on gene mutations was considered acceptable.

Carcinogenicity

No new carcinogenicity studies were performed in light of the fact that no evidence for carcinogenicity was found in the JMPR summaries, and that new genotoxicity studies with imidacloprid were negative.

The JMPR reported carcinogenicity studies in rats and mice. These studies did not reveal carcinogenic effects. For the study in mice, the JMPR established a NOAEL of 66 mg/kg bw/day, based on reduced weight gain. For the study in rats, the JMPR established a NOAEL of 5.7 mg/kg bw/day, based on an increased incidence of mineralization in the colloid of the thyroid gland follicles in males. The JMPR used this NOAEL as the point of departure for the toxicological ADI. These studies were made available for review by the CVMP, and the CVMP confirmed the evaluation by JMPR. Based on these studies, the CVMP concludes that imidacloprid is unlikely to pose a carcinogenic risk to humans exposed to residues in food at the levels observed.

Studies of other effects including immunotoxicity and neurotoxicity

Imidacloprid was negative in the Magnussen-Kligman maximisation test in guinea pigs, and did not cause skin or eye irritation.

Badgujar et al. (2013) described a delayed-type hypersensitivity and T-cell proliferation stimulation response reduced at a dose of 10 mg/kg bw/day in mice. The NOAEL in this study was 5 mg/kg bw/day. Gawade et al. (2013) published a developmental immunotoxicity study and described effects on immunoglobulin concentrations, haemagglutination and delayed type hypersensitivity at 30 and 90 mg/kg bw/day in rats. Effects were minimal at 10 mg/kg bw/day. The LOAEL in this study was 10 mg/kg bw/day.

In addition, the developmental immunotoxicity of imidacloprid was investigated as part of the new GLP-compliant extended one generation study in the rat described above. In this study, where animals were given dietary imidacloprid at concentrations of 0, 100, 300 or 1000 ppm, a dose-dependent reduction of T-cell dependent antibody response was apparent in females at all doses levels, which did not reach statistical significance due to high variances in the results. Moreover, there was a slight reduction of group average mean cell counts for total T-lymphocytes, helper T-lymphocytes and cytotoxic T-lymphocytes in males. In addition, moderate imidacloprid related changes on organ

weights, macroscopical and microscopical effects were observed in thymus, spleen and lymph nodes at 1000 ppm and red to dark pigmentation in thymus in females from 300 ppm on.

A weight of evidence approach as proposed by the WHO (2012, Harmonization Project Document No. 10 Guidance for Immunotoxicity Risk Assessment for Chemicals) leads to the conclusion that there is evidence of an immunotoxic potential of imidacloprid. Literature data indicated a NOAEL of 5 mg/kg/bw.

In the new extended one generation study in the rat, two cohorts of F1 animals were selected to investigate developmental neurotoxicity and neuropathology, respectively and parental animals and neonates were exposed to imidacloprid at dietary concentrations of 0, 100, 300 or 1000 ppm. There was no evidence of developmental neurotoxicity in this study, and no macroscopic or microscopic abnormalities in the brains of neonates attributable to imidacloprid exposure. The doses in this cohort were up to approximately 120 mg/kg bw/day. Only in one segment of cohort 2 (test for intra-session habituation to the acoustic startle), results could not be evaluated conclusively due to statistical shortcomings. In the new long-term repeated dose oral toxicity study in rats, including standard arena observations and functional observation battery, no signs of neurotoxicity were noted up to 63.7 mg/kg bw/day, the highest dose tested.

From other repeated dose toxicity studies, summarised by the JMPR, it appears that neurological clinical signs can be observed at very high doses (150 mg/kg bw/day and above).

2.1.4. Calculation of the toxicological ADI or alternative limit

The overall NOAEL is 5.25 mg/kg bw/day, based on the reduction of body weights of rats in the EOGRTS. Using the default uncertainty factor of 100, a toxicological ADI of 52.5 µg/kg bw (3150 µg for a 60 kg person) is established.

2.1.5. Overview of microbiological properties of residues

Imidacloprid is not classified as an antimicrobial agent and is not structurally related to antimicrobial agents used in human or animal medicine. Data on microbiological properties are therefore not considered necessary.

2.1.6. Calculation of microbiological ADI

As no microbiological effects are expected, the establishment of a microbiological ADI is not considered necessary.

2.1.7. Observations in humans

Some case studies of intentional or accidental oral exposure to imidacloprid have been published. However, these reports generally lack quantitative information on the oral exposure.

2.1.8. Findings of EU or international scientific bodies

In their 2002 publication, the WHO reported on the imidacloprid safety evaluation by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR). The JMPR concluded the following: "The Meeting established an ADI of 0-0.06 mg/kg bw on the basis of the NOAEL for effects on the thyroid gland of 100 ppm, equal to 5.7 mg/kg bw per day, in the long term study of toxicity and carcinogenicity in rats

and a safety factor of 100. This ADI is supported by the NOAEL for effects on the liver in parental animals in the multigeneration study of reproductive toxicity".

In 2008, the European Food Safety Agency (EFSA) reviewed the safety and residues of imidacloprid[7] in the context of the consumer exposure to residues, following the use of imidacloprid as a plant protection product. The EFSA concluded: "The acceptable daily intake (ADI) was 0.06 mg/kg bw/day based on the chronic rat study, the acceptable operator exposure level (AOEL) was 0.08 mg/kg bw/day based on the 90-day dog study supported by the subchronic rat neurotoxicity study, and the acute reference dose (ARfD) was 0.08 mg/kg bw/day based on the 90-day dog study. All reference values were derived with a safety factor of 100".

In 2013, the EFSA Plant Protection Products and their residues (PPR) Panel reviewed the neurotoxicity of imidacloprid[8]. This review was done because of a concern raised by the results of the *in vitro* study published by Kimura-Kuroda *et al*. in 2012. Whereas the Panel raised concerns over the potential for developmental neurotoxicity, it also concluded that "…the current ADI for imidacloprid is considered adequate to protect against its potential developmental neurotoxic effects".

In 2018, imidacloprid was once again reviewed by the EFSA, with a focus on the toxicity for bees[9].

In 2019, the EFSA reviewed the residues of imidacloprid in the context of consumer exposure to residues in plants, processed commodities, rotational crops and livestock following the use of imidacloprid as a plant protection product. However, the EFSA did not re-evaluate the safety in this procedure.

The CVMP notes that the JMPR and EFSA have reviewed the same data and arrived at the same ADI of 0.06 mg/kg bw. In addition, the CVMP notes that the concerns over the potential developmental neurotoxicity have now been adequately addressed by the new extended one generation toxicity study (EOGRTS). New data that became available after the safety evaluations by JMPR and EFSA result in a different overall NOAEL/LOAEL.

2.1.9. Overall conclusions on the ADI

A pharmacological or microbiological ADI is not considered necessary for this substance. The toxicological ADI of 52.5 µg/kg bw is therefore the overall ADI for the risk assessment of imidacloprid.

2.2. Residues assessment

The MRL application relates to the use of imidacloprid in *Salmonidae*. Salmon is a major food producing species. For this target species, a radiolabelled metabolism study was provided, and a cold residue depletion study at different water temperatures. In addition, residue depletion data in rainbow trout were provided, in order to establish the MRL for *Salmonidae* (which includes both salmon and trout). The studies were conducted under GLP and in accordance with relevant guidelines.

2.2.1. Pharmacokinetics in target species

In a GLP-compliant radiolabel study, 26 Atlantic salmon were given a bath treatment with ^{14}C imidacloprid in seawater at 7 °C at a concentration of 20 mg/l for a duration of 60 minutes. Samples of muscle + skin (in natural proportions), liver, spleen, gut, gills and kidney were collected from groups

[7] https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2019.5570
[8] https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2018.5306
[9] https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2018.5178

of 6 fish at 5 hours, 25 hours, 5 and 26 days post treatment. In sub samples the total radioactivity was measured using standard liquid scintillation counting (LSC) analysis. The nature of the residues was determined using a LC-MS method. The radioactive residues in muscle plus skin declined from 359 µg equivalents/kg at 5 hours, to 329, 182, and 13 µg equivalents/kg at 25 hours, 5, and 26 days, respectively. The major part of the detected radioactivity was the parent compound, accounting for 69.4-95.2% of the total radioactive residue in muscle plus skin, and 77.7-95.2% in liver. The only metabolite identified via LC-MS analysis, in all extractable residues was the hydroxy derivative of imidacloprid (representing a maximum of 8.2% TRR) but it was not clear if this was the 4- or 5-hydroxy compound.

2.2.2. Residue depletion studies

Depletion in tissues

In a GLP-compliant residue depletion study, Atlantic salmon were treated with imidacloprid, using a water bath immersion at a concentration of 20 mg/l for 60 minutes at 7°C or 15°C. Groups of 10 fish were slaughtered at days 1, 7, 21, 35 and 60 for the 7°C exposure, and at days 1, 7, 14, 21 and 28 for the 15°C exposure. Samples of liver, muscle, skin, and muscle plus skin in natural proportions were taken and analysed for imidacloprid using a fully validated LC-MS/MS method. The residues in muscle were somewhat higher than the residues in skin. Following exposure at 7°C, mean imidacloprid concentrations in muscle + skin declined from 124 µg/kg at day 1, to 61.2 µg/kg at day 7, 11.75 µg/kg at day 21, 5.29 µg/kg at day 35, and to below the LOQ of 4 µg/kg at day 60. When the exposure occurred at 15°C, mean imidacloprid concentrations in muscle + skin declined from 302 µg/kg at day 1, to 50.0 µg/kg at day 7, 12.58 µg/kg at day 14, and to below the LOQ of 4 µg/kg at days 21 and 28. The study demonstrated an initial higher absorption and a faster depletion at the higher temperature. The bodyweights of fish treated at 15 °C were significantly lower than those of fish treated at 7 °C, however this is of minor significance for the establishment of an MRL.

A study to investigate the residues depletion of imidacloprid in both salmon and trout under commercial treatment conditions, indicated that the residue levels and residue depletion of imidacloprid in salmon and trout are very similar.

Selection of marker residue and ratio of marker to total residues

Because the parent imidacloprid accounted for more than 90% of the total residues in muscle and skin from salmon, the parent imidacloprid can be used as the marker residue with a ratio of marker to total residues of 0.9. This ratio was determined at 7°C, which is considered a relevant temperature.

2.2.3. Monitoring or exposure data

No monitoring or exposure data were available.

2.2.4. Analytical method for monitoring of residues

An analytical LC-MS/MS method was developed and validated for the determination of imidacloprid in salmon and trout muscle and skin in natural proportions. The method is presented in an internationally recognized format and has been validated in line with the current requirements of Commission Regulation (EU) 2018/782 establishing the methodological principles for the risk assessment and risk management recommendations referred to in Regulation (EC) No 470/2009. The validated limit of quantification in muscle plus skin is 4 µg/kg.

The relevant European Reference Laboratory (EURL) has reviewed the analytical method and is in agreement with the above assessment.

2.2.5. Potential effects on the microorganisms used for industrial food processing

As the substance is not expected to possess antimicrobial activity no effects on microorganisms used for industrial food processing are expected. Furthermore, the substance is not intended for use in dairy animals and therefore potential effects in dairy products were not investigated.

2.2.6. Findings of EU or international scientific bodies

The EFSA has calculated the consumer exposure to imidacloprid from crops and livestock (that have been fed with crops containing residues) following the use of imidacloprid as a plant protection product[10]. This use is nowadays limited to indoor use only, in view of the risks for bees when using outdoors. The exposure calculation, that additionally took into account the import tolerances, was based on WHO cluster diet B and resulted in an intake of 6% of the ADI (as established by the EFSA).

Imidacloprid was also approved by ECHA for use in biocides. The ECHA assessment indicates that biocidal use of imidocloprid is not expected to result in residues in food.

3. Risk management recommendations

3.1. Availability of alternative medicines and other legitimate factors

Availability of alternative medicines

Several alternative veterinary medicines, representing various active substances, for the treatment of sea-lice infections on Atlantic salmon (*Salmo salar*) or Atlantic salmon and rainbow trout (*Oncorhynchus mykiss*) are available in the EU. The products have been approved nationally or in MRP/DCP. Medicinal products for the treatment of sea-lice infections on Atlantic salmon are available as bath treatments or in-feed treatments. Emerging resistance development is seen in sea-lice to many of the medicinal products available.

Feasibility of controls

Residues have been studied in muscle and skin of the target species. Muscle and skin (in natural proportions) is therefore the appropriate target tissue for monitoring residues.

No other relevant factors were identified for consideration of the risk management recommendations.

[10] https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2019.5570

3.2. Elaboration of MRLs

Based on the residue depletion data available in salmon and trout, and the ratio of marker to total residues of 0.9, an MRL value of 600 µg/kg for muscle plus skin in natural proportions can be derived.

Calculation of theoretical daily intake of residues

Based on an MRL of 600 µg/kg, a ratio of marker to total residues of 0.9, and a standard food factor of 300 g fish muscle and skin per day, a maximum theoretical daily intake of 200 µg is calculated. This would represent approximately 6% of the ADI.

EFSA reported that the use of imidacloprid as a plant protection agent may result in 6% of the ADI established by EFSA (which is similar to the ADI established by CVMP). ECHA reported that use of imidacloprid in biocidal products is not expected to result in residues in food.

Considering the use of imidacloprid as a plant protection product and as a veterinary medicinal product together, a worst-case daily exposure would not exceed 12% of the ADI.

4. Considerations on possible extrapolation of MRLs

The MRL application relates to the use of imidacloprid in *Salmonidae*. Salmon is a major food producing species. For this target species, a radiolabel metabolism study was provided, and a cold residue depletion study at different water temperatures. In addition, residue depletion data in rainbow trout were provided, in order to establish the MRL for *Salmonidae* (which includes salmon and trout).

Based on the data available, the CVMP concludes that extrapolation to all fin fish is possible.

Extrapolation from *Salmonidae* to terrestrial species is not possible in accordance with Commission Regulation (EU) 2017/880.

5. Conclusions and recommendation for the establishment of maximum residue limits

Having considered that:

- the toxicological ADI of 52.5 µg/kg bw (i.e. 3150 µg/person) was established as the overall ADI for imidacloprid;
- imidacloprid was retained as the marker residue;
- the ratio of marker to total residues was 0.9 in salmon muscle and skin in natural proportions;
- a validated analytical method for the determination of imidacloprid in salmon and trout muscle and skin was available indicating that residues in edible tissues can be adequately monitored in fish;
- maximum residue limits established in *Salmonidae* can be extrapolated to all fin fish;

the Committee recommends the inclusion of imidacloprid in table 1 of the Annex to Commission Regulation (EU) No 37/2010 in accordance with the following table:

Pharmaco-logically active substance	Marker residue	Animal species	MRLs	Target tissues	Other provisions	Therapeutic classification
Imidacloprid	Imidacloprid	Fin fish	600 µg/kg	Muscle and skin in natural proportions	NO ENTRY	Antiparasitic agents / Agents against ectoparasites

Based on the recommended maximum residue limit the theoretical intake of residues from fish tissues represents approximately 6% of the ADI based on a ratio of marker to total residues at 7 °C. The low utilization of the ADI is expected to provide a sufficient margin to compensate for possibly lower ratios of marker to total residues at higher temperatures. The possible consumer exposure to imidacloprid from its use as a plant protection product is also approximately 6% of the ADI. Therefore, the total daily exposure from both sources will not exceed 12% of the ADI.

6. Background information on the procedure

Submission of the dossier	23 April 2019
Steps taken for assessment of the substance	
Application validated:	15 May 2019
Clock started:	16 May 2019
List of questions adopted:	12 September 2019
Consolidated response to list of questions submitted:	17 January 2020
Clock restarted:	20 January 2020
Ad-hoc expert group meeting on genotoxicity:	13 March 2020
List of outstanding issues adopted:	18 March 2020
Response to list of outstanding issues submitted:	8 July 2020
Clock restarted:	12 August 2020
Opinion adopted:	9 September 2020