Bundesinstitut für Risikobewertung

DOI 10.17590/20211105-095751

# Marking Eels with Alizarin Red S: Additional Studies Needed to Assess Health Risks

BfR opinion No 034/2021 of 5 November 2021

To stabilise stocks of European eel, young eels are caught on the Atlantic coast and then re-released in inland waters. The eels are released either immediately (glass eel stock) or after rearing in eel farms (farm eel stock). Before the eels are released, they are often marked with the dye Alizarin Red S (ARS) in order to better monitor the success of the measures being taken.

The dye is primarily stored in bony structures and can be detected in recaptured eel stocks even years later. The German Federal Institute for Risk Assessment (BfR) has investigated the consumption of marked eels as well as the health risks that may result from this. Based on current knowledge, however, a risk assessment is not possible. Information is lacking regarding the estimation of the daily intake of ARS by certain consumer groups who may regularly consume marked eel, as well as additional data on the assessment of the toxicological properties of ARS.

In the opinion of the BfR, only a preliminary assessment of the toxicological relevance of ARS residues possibly occurring in edible eel tissue can be made at the present time. This assessment was based on the so-called TTC concept. Substances are divided into different categories based on their chemical structure. For each of these categories, a so-called *Threshold of Toxicological Concern* (TTC) was previously established on the basis of empirical data. The TTC describes the maximum daily intake of a substance at which, according to the current state of knowledge, it can be assumed that health impairments are unlikely to occur.

The BfR experts interpreted certain features in the chemical structure of ARS as an indication of a possible genotoxic activity. Therefore, a TTC of 0.0025 µg/kg bw (micrograms per kilogram of bodyweight) was used as the basis for assessing toxicological relevance. In addition, it was assumed that the eel muscle tissue contained an ARS concentration equal to the detection limit of 8.9 µg/kg. The model calculations carried out on this basis showed that, in the long-term, a person could consume an average of up to 0.28 g of eel per day per kg of bodyweight without exceeding the TTC. However, it was believed that at least certain consumer groups may consume more than 0.28 g of eel per day per kg of bodyweight over a longer period of time. This would lead to the TTC value for genotoxic (mutagenic) substances being exceeded. However, these calculations are subject to considerable uncertainty. This provisional assessment of toxicological relevance using the TTC concept does not constitute a conclusive risk assessment. Additional studies are required for health risk assessment.

## 1 Subject of the Assessment

The German Federal Institute for Risk Assessment (BfR) publishes its opinion on possible consumer health risks associated with the consumption of eels marked with Alizarin Red S (ARS). The dye Alizarin Red S (ARS) is used to mark young eels (glass eels or farm eels) in order to control the success of re-stocking initiatives. The dye is primarily stored in bony structures and can still be detected even years later. The present assessment was carried out on the occasion of the publication of new data on the accumulation of ARS in edible eel muscle tissue (Baer *et al.* 2020; Kullmann *et al.* 2020), which had been collected as part of a

research project by the State Research Centre for Agriculture and Fisheries of Mecklenburg-Western Pomerania, the State Office for Agriculture, Food Safety and Fisheries of Mecklenburg-Western Pomerania and the University of Hamburg.

## 2 Results

The data currently available are not sufficient for carrying out a conclusive risk assessment. For a comprehensive scientific assessment of a substance that is to be intentionally introduced into the food chain, comprehensive data on its toxicological properties and resulting exposure are usually required. Section 3.2.2 of this Opinion explains in detail which data, from the BfR's point of view, would be necessary for a conclusive scientific risk assessment in this specific case.

A provisional assessment of toxicological relevance is possible using the TTC concept. The provisional assessment based on the TTC (*Threshold of Toxicological Concern*) does not, however, constitute a risk assessment, but could be used as a parameter in risk management decision-making in this particular case.

As ARS shows indications of genotoxic potential due to its structural similarity to known genotoxic substances and, in addition, a potential exposure above the corresponding TTC value appears possible, at least among certain consumer groups, the BfR believes this endpoint should be clarified experimentally (more detailed information in Section 3.2.1).

If ARS turns out to be genotoxic in the *in vitro* studies and this finding is also confirmed *in vivo*, the intended introduction of the substance into the food chain would not, from the BfR's point of view, comply with the minimisation approach usually applied for genotoxic and (potentially) carcinogenic substances (ALARA principle; *as low as reasonably achievable*). If, however, the new studies do not corroborate the suspicion of genotoxicity, the probability of the occurrence of adverse health effects would be considered as low according to the TTC concept. However, due to a high level of uncertainty, this assessment would not represent a conclusive risk assessment either. Approval of ARS for marking in fishery biology projects is a risk management issue.

## 3 Rationale

### 3.1 Results from Previous BfR Assessments

The BfR first dealt with an assessment of the possible consumer health risks from the consumption of eels marked with ARS in 2016. From the BfR's point of view, a conclusive risk assessment was not possible at that time. The reasons for this were, on the one hand, the lack of toxicological data to characterise the hazard potential of ARS. On the other hand, exposure to ARS resulting from the consumption of marked eels could not be evaluated, as no data on the respective levels in edible eel tissues and no reliable data on eel consumption were available.

In 2019, the BfR again dealt with the assessment of health risks associated with the use of ARS to mark eels. For this purpose, new data on the accumulation of the dye in edible eel muscle tissue were taken into account, which had been collected as part of a research project by the State Research Centre for Agriculture and Fisheries of Mecklenburg-Western Pomerania, the State Office for Agriculture, Food Safety and Fisheries of Mecklenburg-Western Pomerania and the University of Hamburg and were forwarded to the BfR via the Federal Ministry of Food and Agriculture (BMEL).

The new information on the accumulation of ARS in eel muscle tissue only permitted a preliminary assessment based on the TTC concept. The chemical structure of ARS shows certain characteristics that can be interpreted as an indication of genotoxic potential. Therefore, a TTC of 0.0025 µg/kg bw (bodyweight) was taken into account in the preliminary assessment. In addition, it was assumed that the eel muscle tissue contained an ARS concentration equal to the analytical detection limit of 8.9 µg/kg ("upper bound approach"). The model calculations carried out on this basis showed that, in the long-term, a person could consume an average of up to 0.28 g of eel per day per kg of bodyweight without exceeding the TTC for substances with genotoxic potential (0.0025 µg/kg bw). However, on the basis of the available data, it was believed that at least certain consumer groups may consume more than 0.28 g of eel per day per kg of bodyweight over a longer period of time.

However, due to the lack of substance-specific data, these model calculations are subject to considerable uncertainties. A provisional assessment using the TTC concept should not be understood as representing a conclusive risk assessment. The BfR points out that the TTC concept was originally established for assessing the toxicological relevance of unintended or unavoidable exposure to contaminants.

An updated search of the scientific literature on ARS performed by the BfR did not reveal any relevant new information beyond those of the aforementioned publications on ARS residues in fish (Baer *et al.* 2020; Kullmann *et al.* 2020).

### 3.2 Recommendations for Improving the Data Basis

The BfR is not aware of any specifications for scientific risk assessment that directly relate to the use of chemical substances to mark animals used for food production.

In order to answer the question of which tests would be required to assess the risk of using ARS as a marker substance within fisheries biology measures, the present opinion refers to existing guidelines that set out requirements for similar issues in the food sector within the EU. Before food and feed additives or veterinary medicinal products, for example, can be introduced into the food chain, a full risk assessment must be undertaken to demonstrate the absence of health risks to consumers. This requires the formulation of certain minimum requirements with regard to the toxicological data needed for the scientific assessment, which are usually specified by legal regulations and scientific guidelines. Such requirements are, for example, contained in the guidelines of the European Food Safety Authority (EFSA) for the assessment of food additives (*"Guidance for submission for food additive evaluations*") or for the assessment of animal feed additives (*"Guidance on the assessment of the safety of feed additives for the consumer"*) (EFSA 2012; EFSA 2017).

With regard to contaminants, the requirements for risk assessment are less clearly defined. In this context, the EFSA Scientific Committee recommends the use of the TTC concept as a decision-making tool to assess whether the exposure to such substances is sufficiently low to regard the occurrence of adverse health effects as being unlikely (EFSA 2019). If the TTC is exceeded, additional data are usually required for a health risk assessment (EFSA 2019).

Approval of ARS as a marker substance for fisheries biology measures is a risk management issue. From a scientific assessment standpoint, two possible options exist for action in this particular case, which are explained in more detail below.

### 3.2.1 Provisional Assessment of Toxicological Relevance Using the TTC Concept

In the TTC concept, substances for which no or insufficient toxicological data are available are assigned to a substance class on the basis of their chemical structure. On the basis of extensive toxicological data on structurally related compounds, maximum intake levels (TTC) were derived for each of these substance classes, up to which the probability that they would cause adverse health effects is regarded as low.

The application of the TTC concept is only intended for a preliminary assessment of the toxicological relevance of unintended or unavoidable exposure. More extensive data are usually required for a toxicological risk assessment. For substances that are intended to be introduced into the food chain and are subject to an authorisation procedure within the EU, an assessment based on the TTC concept alone is considered insufficient due to the uncertainties involved. However, the provisional assessment based on the TTC could, in this particular case, be used as a parameter in risk management decision-making.

In 2019, using the TTC concept, the BfR provisionally described the toxicological relevance of the exposure to ARS that may have resulted from the consumption of marked eels. Because ARS is structurally similar to known genotoxic compounds, a TTC of 0.0025 µg/kg bw was taken into account. In addition, it was assumed that the eel muscle tissue contained an ARS concentration equal to the reported detection limit of 8.9 µg/kg (*"upper bound approach"*). The model calculations carried out on this basis showed that, in the long-term, a person could consume an average of up to 0.28 g of eel per day per kg of bodyweight without exceeding this TTC for substances with genotoxic potential (0.0025 µg/kg bw). For a person weighing 60 kg, this corresponds to an average consumption of 17 g of eel per day. However, it was believed that at least certain consumer groups may consume more than 0.28 g of eel per day per kg of bodyweight over a longer period of time. This would lead to the TTC value for genotoxic substances being exceeded.

In case of structural indications of genotoxic potential and exposure levels that could lead to the corresponding TTC value being exceeded, toxicological studies are usually required to clarify the genotoxic potential (EFSA 2019). The BfR therefore believes that it is necessary to clarify genotoxic potential of ARS experimentally.

According to the EFSA guideline "*Scientific opinion on genotoxicity testing strategies applicable to food and feed safety assessment*", the following studies, for example, are suitable for an assessment as an initial step (EFSA 2011):

- *"Bacterial Reverse Mutation Assay"* according to OECD TG 471 to clarify gene mutagenic potential

and in addition, at least

- *"In vitro Mammalian Cell Micronucleus Test"* on mammalian cells according to OECD TG 487 to clarify clastogenic and aneugenic potential

The studies should be carried out under accredited conditions and in accordance with the standards of "Good Laboratory Practice" (GLP). If both tests are negative, it may be assumed that the substance under assessment possesses no genotoxic activity. Positive findings, on the other hand, should be further clarified with the help of suitable *in vivo* studies (EFSA 2011).

If ARS turns out to be genotoxic in the *in vitro* studies and this finding is also confirmed *in vivo*, the intended introduction of the substance into the food chain would not, from the BfR's point of view, comply with the minimisation approach usually applied for genotoxic and (potentially) carcinogenic substances (ALARA principle: *as low as reasonably achievable*).

If, however, the new studies do not corroborate the suspicion of a genotoxic effect, ARS would have to be assigned to Cramer class III according to the extended decision rules of Cramer *et al.* (1978) implemented in the Toxtree software (v2.6.13) (TTC: 1.5 µg/kg bw). In this case, the occurrence of harmful effects according to the TTC concept should be assessed as unlikely.

The BfR points out that reliable new data on the genotoxicity of ARS would only eliminate part of the existing uncertainties. The data on the potential residues of the dye substance in the edible tissue of adult eels are also subject to a high level of uncertainty. The examinations carried out by Kullmann *et al.* (2020) do not correspond to current standards used for determining residues in animal tissues, such as those required for animal feed additives (EFSA 2017). On the one hand, no radioactively labelled substances was used, which means that no conclusions can be drawn on any metabolites that may be formed. Moreover, the BfR has no information on chemical contaminants of the dye substances used. This could lead to a considerable underestimation of the toxicological relevance. On the other hand, the model calculations were based on the assumption that ARS is present in the edible eel tissue at the level of the detection limit. This could result in overestimation of the toxicological relevance.

### 3.2.2 Data Requirements as Part of a Comprehensive Assessment of Possible Health Risks

The data requirements considered necessary for a risk assessment may differ depending on the intended use of a substance. As a rule, however, certain minimum requirements are set in order to be able to carry out a sufficiently reliable risk assessment. In the opinion of the BfR, the following information is essential in order to be able to carry out a scientific assessment of the health risks for consumers:

(1) *Chemical data*

- Substance identity
- Physicochemical properties of the substance
- Specification of the dye substance in terms of purity and impurities

(2) *Exposure estimation data*

- Absorption, distribution, metabolism and excretion (ADME) of the dye substance in marked fish
- Residues from the dye solution in edible tissues at the time of food production
- Representative data on the consumption of food obtained from marked fish

The studies with regard to the residues should in principle meet the requirements as described, for example, for feed additives (EFSA 2017), and also take into account relevant metabolites and impurities in the dye substance used. If these studies indicate a very low level of consumer exposure, a risk assessment could also be carried out without consumption data.

(3) *Toxicological data*

The required toxicological studies relate to all residues for which a relevant exposure is assumed and which, taking into account all available information, are assessed as toxicologically relevant.

(a) *Characterisation of the genotoxic potential*

In accordance with the basic assumption that a threshold dose cannot be determined for genotoxic substances with a DNA-reactive mechanism of action and that these substances should therefore not be intentionally introduced into the food chain, the following *in vitro* tests would be suitable for an initial assessment of genotoxic activity.

- *"Bacterial Reverse Mutation Assay"* according to OECD TG 471 to clarify gene mutagenic potential

and in addition, at least

- *"In vitro Mammalian Cell Micronucleus Test"* on mammalian cells according to OECD TG 487 to clarify clastogenic and aneugenic potential

Positive findings would then have to be further clarified using suitable *in vivo* studies in accordance with the recommendations of the EFSA (EFSA 2011).

(b) *Characterisation of the substance's inherent toxic properties*

In addition to clarifying genotoxic potential, general characterisation of the toxic properties inherent to the substance under assessment must also be carried out. According to the relevant EFSA guidelines, this generally requires at least one subchronic toxicity study (EFSA 2012; EFSA 2017):

- *"Repeated Dose 90-Day Oral Toxicity Study in Rodents*" according to OECD TG 408 to clarify possible toxic properties and derive toxicological guidance values, such as an *acceptable daily intake* (ADI)

(c) *Additional characterisation of the substance's inherent toxic properties*

Depending on the results of the genotoxicity test and the subchronic toxicity study, additional studies (e.g. chronic oral toxicity, reproductive toxicity, carcinogenicity) may be required for a final assessment.

**Further information on the BfR website**

Subject page on fish:
https://www.bfr.bund.de/en/a-z_index/fish-129987.html

BfR 'Opinions app'

## 4 References

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### About the BfR

The German Federal Institute for Risk Assessment (BfR) is a scientifically independent institution within the portfolio of the Federal Ministry of Food and Agriculture (BMEL) in Germany. The BfR advises the Federal Government and the States (‘Laender’) on questions of food, chemical and product safety. The BfR conducts its own research on topics that are closely linked to its assessment tasks.

*This text version is a translation of the original German text which is the only legally binding version.*