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To the Inspector-General of the Netherlands Food and Consumer Product Safety Authority (NVWA) and the Minister of Health, Welfare and Sport

Advice from the Director of the Office for Risk Assessment and Research (BuRO)

The health risks of environmental contaminants in wilderness meat from floodplains.

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Background

At the beginning of 2020, the Netherlands Food and Consumer Product Safety Authority (NVWA) received two reports on consumers' health risks in relation to so-called wilderness meat. Unlike regular[1] beef, this wilderness meat comes from specific breeds of cattle such as Rode Geus, Galloway, Tauros and Scottish Highland cattle that are used year-round for natural grazing in the floodplains and other nature reserves. Surplus animals that cannot be rehomed to other areas are slaughtered and operators sell their meat to consumers. In response to the reports, the NVWA's Inspection Directorate initiated a study into the presence of dioxins and dioxin-like polychlorinated biphenyls (PCBs) in wilderness meat from four[2] Dutch floodplains. Some of the liver and meat samples that had been analysed showed that the level of dioxins and PCBs exceeded the statutory maximum levels (MLs).

Following these findings, the Inspection Directorate asked the Office for Risk Assessment & Research (BuRO) a number of questions pertaining to possible consumer health risks and the interpretation of the results that had been obtained. These subsidiary questions relate to the following main question:

Could the presence of dioxins and dioxin-like PCBs in wilderness meat from the floodplains lead to risks to consumers' health?

BuRO concluded that there was insufficient information available to answer this question, for which reason it commissioned additional research. In this process, the question was also expanded to include two other relevant groups of environmental contaminants: heavy metals[3] and poly- and perfluoroalkyl substances (PFASs).

Approach

BuRO commissioned Wageningen Food Safety Research (WFSR) and the National Institute for Public Health and the Environment (RIVM) to conduct additional research. WFSR examined tissues of wild cattle from a number of floodplains along the major rivers and measured the levels of dioxins, dioxin-like PCBs, non-dioxin-like PCBs, PFASs and heavy metals in wilderness meat (Hoogenboom et al., 2022b). WFSR also collected grass and soil samples at a number of these locations and measured the levels of these contaminants(Hoogenboom et al.,

[1] Cattle kept as farm animals.
[2] Regarding the floodplains at Loevestein, Millingen, Border Meuse and Kaliwaal.
[3] Cadmium, lead, mercury, copper, nickel and metalloid arsenic.

2022b). Based on a first set of analysis results of dioxins, RIVM, in collaboration with WFSR, commissioned by BuRO, developed a transfer model to estimate the transfer from dioxins and dioxin-like PCBs from soil and grass to these bovine animals (Minnema et al., 2021; Notenboom et al., 2021). On the basis of the levels in soil and grass, this model can estimate the levels in wilderness meat. WFSR subsequently performed additional measurements for dioxins and dioxin-like PCBs and PFASs in the tissue and plasma of bovine animals to gain more insight into the seasonal factors and the rate at which levels of dioxins and PFASs levels can be reduced by rehoming the animals to a clean environment (Hoogenboom et al., 2022a). Finally, on behalf of BuRO, the RIVM/WFSR Food and Product Safety Front Office (FO) made inquiries into data that could provide insight into consumer consumption of wilderness meat (Front office, 2021).

BuRO has drawn up this advice on the basis of abovementioned research and literature review[4]. Where possible, the intake of the substances tested from wilderness meat was compared with intake from other meats and intake from the total Dutch diet.

Present advice describes the assessment of the consumers' health risks in relation to dioxins and dioxin-like PCBs, heavy metals and PFASs in wilderness meat. The substantiation details the risk assessment of these substance groups separately (parts 1 to 3).

Findings

<u>Hazard identification and characterisation</u>

- When grazing in the floodplains, bovine animals ingest grass, soil particles, suspended river silt and surface water that contain environmental contaminants. The levels of environmental contaminants in soil and grass may be higher in floodplains than other areas due to disposal of river silt and sediment. The present research focuses on dioxins and dioxin-like PCBs, heavy metals and PFASs.

Dioxins and dioxin-like PCBs

- Dioxins and dioxin-like PCBs are persistently present in the environment and, after intake, accumulate in the liver and fat of animals and humans. Increased levels of soil and grass may be caused by the disposal of river silt and sediment and by local atmospheric deposition in combustion processes. People may be exposed to dioxins via food, especially from eating fat animal products such as fish, meat, milk and eggs. A number of MLs have been established at European level for dioxins, PCBs and dioxin-like PCBs that animal products must comply with.
- A long-term exposure to dioxins and dioxin-like PCBs can lead to adverse human health effects. Male reproductive toxicity is the critical toxicological effect. EFSA has established a Tolerable Weekly Intake (TWI) as a health-based guidance value of 2 pg WHO-TEQ[5]/kg bodyweight/week. Non-dioxin-like PCBs have different toxicological properties and have not been included in this risk assessment due to the lack of a health-based guidance value.

Heavy metals

- Heavy metals occur naturally in soil and may be present in elevated concentrations as a result of sedimentation of contaminated silt or as a result

[4] The search strategy is described in Appendix 1.
[5] Toxic equivalents, i.e. the weighted sum of individual dioxins and dioxin-like PCBs in a mixture.

of regional atmospheric deposition from industrial activities. After intake, metals in particular, accumulate in the animals' organs. Excessive intake of heavy metals can lead to several adverse health effects in humans. MLs have been established at European level for a number of metals that animal products must comply with. Bovine kidneys older than two years may not be offered for consumption in the Netherlands due to the high levels of cadmium.

PFASs

- PFASs are man-made chemical and thermally-stable substances, which remain present in the environment and the food chain for long periods of time and some of which accumulate in humans and animals. After intake, PFASs primarily accumulate in liver and blood. MLs have since been established for the presence of PFASs. These MLs are expected to enter into force in 2023.
- EFSA has derived a TWI of 4.4 ng/body weight per week for the sum of four PFASs[6]: PFOA, PFOS, PFNA and PFHxS (EFSA-4). EFSA concludes that the effect on the immune system is the critical effect, based on a lower vaccination response in children.
- In applying the TWI, EFSA assumes equipotency; equal potency of each of the EFSA-4 with respect to the toxicological effect on the immune system (concentration addition). According to EFSA's analysis, there were no appropriate studies available to derive relative potency factors (RPFs). RIVM did derive RPFs based on data about liver toxicity (RPF method). The RPFs indicate the toxic potency of individual PFASs relative to the PFOA substance component for different types of PFASs and more than the four PFASs that could be assessed by EFSA. As there is no scientific consensus yet on the application of RPFs for PFASs, BuRO uses the methods of EFSA and RIVM alongside each other.

Exposure estimate

- Wilderness meat is sold year-round. The animals are also slaughtered year-round, though less often in the period between March and June.
- Wilderness meat is predominately sold in frozen packages of different types of meat. The offer consists of lean meat (≤2% fat) plus higher-fat meat products such as minced meat, burgers, chopped steak and sausages (14% fat on average). Offal and fat are no longer being offered by the operators. The meat in one package comes from multiple animals originating from the same area.
- The market share of wilderness meat is small, given the 70,000 kg and about 4,000 private customers. There are consumers, mostly families, who eat this meat twice a week all year round. Based on the FO assessment on the offer and consumption of wilderness meat, BuRO assumes a weekly consumption for this group of 110 grams (average amount) and 300 grams (high consumption amount) per person per week.
- Part of the meat is sold as regular beef through other channels.

Dioxins and dioxin-like PCBs

- A large amount (more than 70%) of the levels of dioxins and dioxin-like PCBs in kidney fat[7] and liver of the cattle tested in fourteen Dutch floodplains turned out to exceed the MLs. However, the operators do not offer the organs of these bovine animals for consumption purposes. The level of dioxins in kidney fat is assumed to correspond with the level in meat fats, for which a risk assessment has been carried out.

[6] PFOS: perfluorooctane sulphonic acid; PFOA: perfluorooctane acid; PFNA: perfluorononane acid and PFHxS: perfluorohexane sulphonic acid.
[7] Kidney fat is a matrix that can also be sampled when animals are lean and therefore have low body fat. The sampling of this matrix was selected for technical reasons.

- Levels of dioxins in kidney fat varied between 1.7 and 59.5 pg TEQ/g fat. The levels were higher on average among young cattle and bulls. Contributing factors may include high exposure of calves via milk, growth dilution in older animals, lower body fat in bulls and milk clarification by lactating cows.
- The levels of dioxins and dioxin-like PCBs in soil and grass in floodplains were higher than in areas outside the floodplains. Floods temporarily increase the levels in grass by deposition of contaminated silt.
- Compared to levels in regular beef, these animals had high levels of dioxins and dioxin-like PCBs. This may be explained by the fact that wild cattle grazes in the floodplains all year round, resulting in continuously higher exposure to environmental contaminants than animals grazing in pastures and/or stabled cattle, especially in the winter months.
- Wild cattle from the floodplains at Loevestein that had been slaughtered in April 2021 had extremely high levels of dioxins in their livers and kidney fat, i.e. 54-59.5 pg TEQ/g fat. The levels of dioxins in animals from this area that had been slaughtered in June 2020 and November 2021 were significantly lower (7.6-28.9 and 2.1-5.1 pg TEQ/g fat respectively). This finding may be indicative for seasonal factors on the level of dioxins in fat.
- RIVM has developed a transfer model for dioxins and dioxin-like PCBs to simulate the transfer of these substances in soil and grass to meat fat and livers of the Rode Geus. The model has been validated with the limited data available on the floodplains of Beuningen (Gelderland). Based on this validation, BuRO considers that the current model can be applied to the simulation of trends in the levels of dioxins during the year. It needs further fine-tuning and validation to quantitatively estimate the transfer.
- The simulation of trends clearly show seasonal effects on levels of dioxins and dioxin-like PCBs in fat and liver due to variation in body weight and variation in levels of contaminants in grass during the year, partly caused by suspended silt. The lowest levels of fat and liver are estimated for late summer and fall, which corresponds to the limited measurements available. The simulations also show that these levels drop below the ML in a period of several months if the animals are rehomed to a clean stable or pasture.
- Measurements in blood and kidney fat show that relocating the animals with high levels of dioxins to a stable can lead to a substantial drop in levels of dioxins in the animals in the course of a few months, mainly through reduction in exposure and through growth and fat deposition, and to a lesser extent through excretion. Rehoming cattle to clean pastures could also, to a lesser extent, lead to reductions in levels of dioxins, provided no local contaminants are present there. BuRO has not carried out any research into the practical feasibility of such measures and the effects they may have on animal welfare.

Heavy metals

- Heavy metal concentrations were most prominent in bovine organs. A number of kidney and/or liver samples showed an excess of the ML for cadmium, lead, mercury or copper. However, operators no longer offer the organs of these bovine animals for consumption purposes.
- All levels of cadmium (<0.006 mg/kg) and lead (<0.008 mg/kg) were below the detection limit. For mercury (≤ 0.006 mg/kg) and copper (0.42-1.9 mg/kg), the levels were well below the respective MLs of 0.01 mg/kg and 5 mg/kg. No MLs have been established for nickel and arsenic for beef. Nickel could not be detected (<0.02 mg/kg) and total arsenic was measured at levels between 0.01 and 0.11 mg/kg with an average of 0.04 mg/kg. These levels of arsenic are within the range of levels in meat of other wildlife animals such as deer, boar and roe deer. Due to the absence of data on levels of arsenic in regular beef, it is not clear whether the consumption of wilderness meat leads to a higher exposure to arsenic when compared with regular beef.

- There were no distinct differences between the levels of heavy metals in grass within the floodplains and a monitoring area outside the floodplains. Levels of heavy metals in grass were strongly elevated just after a flood, probably due to deposition of contaminated silt.

PFASs

- PFOS was found in the sampled meat. Levels in meat were much lower than those in liver and kidney. In addition to PFOS, a number of other PFASs were also found in liver and kidneys.
- Approximately half of the measured livers had a higher level than the ML recently established for PFOS and/or PFNA and higher than the ML established for the sum of four PFASs. In wilderness meat, the ML established for PFOS is exceeded in ten of the 51 animals tested.
- The risk assessment uses the P50 levels of the EFSA-4 in the lower-bound scenario[8]. The concentrations are calculated on the basis of both the concentration addition as well as RIVM’s RPF method. As PFOS is only found in wilderness meat, these sum levels in the lower-bound scenario are solely determined by the PFOS levels. The RPF method can principally include 23 PFASs. BuRO also limits itself with the RPF method to the EFSA-4, as the risk in the UB scenario would otherwise be too dependent on the high limits of quantification (LOQs) of the non-detected PFASs in wilderness meat.
- The levels in the upper-bound scenario[9] are used to determine the uncertainty caused by the possible presence of PFAS levels below LOQ.
- PFAS levels in the lower-bound scenario vary between 0 and 0.94 ng/g (concentration addition) or 0 and 1.88 ng PEQ/g (RPF method). For the upper-bound scenario, the levels of the EFSA-4 are between 0.4 and 1.24 ng/g (concentration addition) or between 1.74 and 3.42 ng PEQ/g (RPF method).
- Based on twelve measurements in the livers of regular bovine animals, which found levels between 0.3 and 1.4 ng/g in liver, the PFOS level in the wild cattle from the floodplains, with levels varying between 1.75 and 65.75 ng/g in liver, appears to be higher than in regular bovine animals.
- A limited number of measurements in meat and liver of cattle from the floodplains of Loevestein showed that PFAS levels were lower after the summer period than after the winter period, presumably due to lower exposure in summer.
- PFOS levels in the blood of wild cattle rehomed to a clean environment can halve in a few months, mainly due to less exposure combined with growth dilution, and to a lesser extent by excretion.

<u>Risk characterisation</u>

Dioxins and dioxin-like PCBs

- For the risk assessment, a number of different exposure scenarios of adult consumers of wilderness meat were tested against EFSA's TWI. The starting point of the risk assessment is that wilderness meat that does not comply with the MLs would still be brought to market, even though this is not permitted. Given that dioxins accumulate in fat, the risk assessment made a distinction between the consumption of wilderness meat products with a higher fat percentage, i.e. an average fat content of 14%, and lean wilderness meat (≤ 2% fat). This shows that the weekly consumption of the meat products with a higher fat content leads to an excessive intake of dioxins and dioxin-like PCBs. Calculated with P50 levels, the TWI is exceeded by a factor of 2.5 at a weekly

[8] The sum of the four PFASs whereby levels of individual PFASs below LOQ are set at zero.
[9] The sum of the four PFAS whereby the levels of individual PFAS below LOQ are made equal to the LOQ.

consumption of 300 grams of meat products. A consumption of 110 grams of meat products does not exceed the TWI, but consumption contributes 90% of the tolerable upper intake levels of dioxins and dioxin-like PCBs. Thus, in this case, there is hardly any room for intake from other sources before exceeding the TWI.

- Children up to 12 kg can only eat 24 grams of meat products with a higher fat content (average of 14% fat) with a P50 level per week before the TWI is exceeded. This is a considerable amount, meaning that health risks can thus not be excluded.
- Consuming lean wilderness meat (≤2% fat) does not lead to the TWI being exceeded for dioxins and dioxin-like PCBs for both children and adults. Lean wilderness meat consumption therefore does not lead to health risks.
- If regular beef is eaten as opposed to wilderness meat, the exposure to dioxins and dioxin-like PCBs is about a factor 40 lower. The TWI is thus not exceeded in any of the exposure scenarios that have been assessed.
- An estimate of the exposure to dioxins and dioxin-like PCBs from the total food consumption of adults and children in the Netherlands, including the consumption of regular beef, shows that this does exceed the TWI. Compared with regular beef, wilderness meat consumption may add to the total exposure, which is already too high.

Heavy metals

- Although heavy metals in the highest concentrations appear in bovine organs, operators no longer offer this offal. It therefore poses no risk to public health.
- The heavy metal levels in meat were below the ML or the LOQ, except for arsenic. Wilderness meat therefore meets the statutory maximum levels applicable to beef for cadmium, lead, copper and mercury.
- The exposure to arsenic from wilderness meat does not in itself lead to health risks.
- Exposure to arsenic from the total Dutch food consumption is high. It is unknown whether the consumption of wilderness meat leads to an increased exposure.

PFASs

- For the risk assessment, a number of different exposure scenarios of adult consumers of wilderness meat were tested against EFSA's TWI. BuRO uses both the EFSA methodology (concentration addition) as well as RIVM's RPF method to determine the PFAS concentration.
- Based on P50 levels and the lower-bound scenario, the risk assessment shows that wilderness meat's highest contribution to the tolerable upper intake levels of PFAS is 50%. This is based on the consumption of 300 grams of wilderness meat per week and the RPFs have been applied for the concentration calculation. PFAS levels in wilderness meat alone therefore do not lead to health risks for the adult consumer, however it does substantially fill up the tolerable upper intake level.
- The consumption of reasonable quantities of wilderness meat exceeds the tolerable upper intake level of PFAS for children up to 12 kg, based on RIVM's RPF method for concentration calculations. This means that the consumption of wilderness meat with P50 levels (lower-bound) could potentially lead to health risks for children. Taking concentration addition, the tolerable upper intake level for children will be filled up for the most part.
- Given that a number of PFASs may have been present in levels below the LOQ, there is an uncertainty in the risk assessment based on the lower-bound scenario. Calculations with the upper-bound scenario show that lower detection limits are needed to definitively assess the risks of high consumption levels (300 grams per week) for adults. The same applies to the intake scenario of children on the basis of concentration addition.
- The indicative calculations from RIVM show that the intake of PFASs from the total Dutch diet (with the consumption of regular beef) and drinking water is too high. This is also apparent from the intake calculations made by EFSA. If wilderness meat indeed contains higher levels of PFASs than regular beef, the consumption of wilderness meat would then contribute to a greater extent to the intake of PFASs.

Answers to the research question

Can the presence of dioxins and dioxin-like PCBs, heavy metals and PFASs in wilderness meat from the floodplains lead to risks to consumers' health?

Further research into environmental contaminants in wild cattle grazing in the floodplains year-round showed that levels of dioxins and dioxin-like PCBs exceeded the ML in over 70% of the samples. The highest levels were found in young animals and animals of the male sex.

BuRO's risk assessment shows that regular consumption of the meat products with a higher fat content (an average fat percentage of 14%) of these bovine animals in the floodplains leads to an excessive intake of dioxins and dioxin-like PCBs, both for adults and for children. Consuming lean meat (≤2% fat) of these bovine animals does not lead to the TWI being exceeded for both children and adults. As the levels of dioxins and dioxin-like PBCs in wilderness meat are higher than in regular beef, wilderness meat consumption may add to the intake from the total diet, which is already too high.

The level of heavy metals in organs exceeded the ML in a number of samples. However, operators no longer offer the organs of these bovine animals for consumption purposes, as far as known. The levels in meat were below the ML or the LOQ. Wilderness meat therefore meets the statutory maximum levels applicable to beef for heavy metals. No ML has been determined for arsenic. BuRO's risk assessment shows that the intake of arsenic alone from wilderness meat does not lead to an elevation of health risks.

The consumption of wilderness meat can lead to an exposure to PFAS of 50% of the TWI, the tolerable upper intake levels for adults. This is based on the consumption of 300 grams of wilderness meat per week and the RPFs have been used for the concentration calculation. The PFAS levels in wilderness meat alone therefore do not lead to the health safety standards being exceeded for adult consumers. Lower detection limits are needed to definitively assess the risks of high consumption levels (300 grams per week) for adults. This uncertainty also applies to the children’s intake scenario based on concentration addition. Based on the data currently available, the consumption of reasonable quantities of wilderness meat appears to exceed the tolerable upper intake level for children up to 12 kg, if RIVM’s RPF method for concentration calculations is used. This means that the consumption of wilderness meat elevates health risks for children consuming lots of meat.

The intake of PFASs from other sources have not been included in this risk assessment. The calculations from RIVM and EFSA show that the intake of PFASs from the total Dutch diet and drinking water exceeds the TWI. This is based on regular beef consumption; the contribution from wilderness meat may be higher.

Variations in levels over time and place
Levels of dioxins and PFASs in wilderness meat vary over time due to variation in the level of exposure, seasonal fat reserves (mainly of relevance to dioxins), growth and excretion, including through milk production. No further research has been carried out into variations in levels for heavy metals. The lowest levels are expected in late summer and fall according to both simulations with a transfer model for dioxins and a limited number of measurements of dioxins and PFASs in bovine animals. Simulations of levels and measurements of dioxins and PFAS levels also show that levels can drop sharply after the (growing) animals have been relocated to a clean environment. BuRO has not carried out any research into the practical feasibility of such measures and the effects on animal welfare if animals were to be rehomed to clean stables or pastures.

Advice from BuRO
To the Inspector-General of the Netherlands Food and Consumer Product Safety Authority (NVWA)
- Do not allow the offer of wilderness meat from bovine animals from Dutch floodplains until it has been demonstrated that the meat from the respective area meets the MLs for dioxins and dioxin-like PCBs.
- Increase the monitoring of PFAS levels in meat from the floodplains and regular beef.
- Notify the operators of large grazers of the factors that play a role in increased exposure of these animals from the floodplains and the possibilities of reducing the levels.

- The operators currently do not offer offal and fat from these animals for consumption purposes. Consider also prohibiting this offer by law.

Yours faithfully,

Prof. Antoon Opperhuizen
Director of the Office for Risk Assessment and Research

SUBSTANTIATION

Background

Wilderness meat comes from specific breeds of cattle such as Rode Geus, Galloway, Tauros and Scottish Highland cattle that are used year-round for natural grazing in the floodplains and other nature reserves. Surplus animals that cannot be rehomed to other areas are slaughtered and their meat sold to consumers. These animals' grazing pattern leads to the animals inevitably coming into contact with environmental contaminants, such as through the intake of soil particles, river silt or surface water. Environmental contaminants, such as dioxins, dioxin-like polychlorinated biphenyls (PCBs), poly- and perfluoroalkyl substances (PFASs) and heavy metals[10], are particularly widespread in the environment due to industrial activities and/or combustion processes. A number of these substances are persistent in the environment and accumulate in the food chain.

In 2020, measurements performed by the Inspection Directorate of the Netherlands Food and Consumer Product Safety Authority (NVWA) showed that a number of liver and meat samples from wild cattle from the four[11] Dutch floodplains contained a level of dioxins and dioxin-like PCBs that exceeded the statutory maximum levels (MLs). At that time, the Office for Risk Assessment & Research (BuRO) concluded that the information available was too limited to draw any conclusions on the presence of environmental contaminants in wilderness meat and the possible consumers' health risks.

BuRO therefore requested Wageningen Food Safety Research (WFSR) to initiate a study to gain more insight into 1) the levels of environmental contaminants in wilderness meat from bovine animals in Dutch floodplains and 2) the local and geographical aspects that play a role (Hoogenboom et al., 2022b). In collaboration with WFSR, the National Institute for Public Health and the Environment (RIVM) then developed a transfer model on behalf of BuRO to model the transfer from dioxins and dioxin-like PCBs from soil and grass to these bovine animals (Minnema et al., 2021; Notenboom et al., 2021). WFSR subsequently took additional measurements for dioxins and dioxin-like PCBs and PFASs in the tissue and plasma of bovine animals to gain more insight into the seasonal factors on levels of dioxins and the rate at which dioxin and PFAS levels can be reduced by rehoming the animals to clean stables or pastures (Hoogenboom et al., 2022a). Finally, the RIVM/WFSR Food and Product Safety Front Office (FO) inquired about data that could provide insight into the consumption of wilderness meat (Front office, 2021). The abovementioned studies and literature review[12] conducted by BuRO form the basis of this advice.

The present advice from BuRO describes the assessment of consumer health risks in relation to the intake of dioxins and dioxin-like PCBs (part 1), heavy metals (part 2) and PFASs (part 3) in wilderness meat consumption.

[10] Cadmium, lead, mercury, copper, nickel and metalloid arsenic.
[11] Regarding the floodplains at Loevestein, Millingen, Border Meuse and Kaliwaal.
[12] The search strategy is described in Appendix 1.

PART 1: DIOXINS AND DIOXIN-LIKE PCBs

Legal framework

Regulation (EC) No 1881/2006[13] sets the European MLs for dioxins, dioxin-like PCBs and indicator PCBs in meat from different types of animals. The levels for bovine animals are summarised in Table 1. The MLs have been established according to the ALARA principle[14]. The MLs have no direct relationship with human health risks. The regulation also contains action levels set to reduce levels of dioxins in animal products[15]. If these action levels are exceeded, the preferred approach would be to identify the source of contamination and take measures to eliminate or reduce the source.

Table 1: MLs of dioxins and dioxin-like PCBs in meat and meat products of bovine animals and bovine liver according to Regulation (EC) No 1881/2006.

Description	**Sum of dioxins (WHO-PCDD/F-TEQ)**	**Sum of dioxins and dioxin-like PCBs (WHO-PCDD/F-PCB-TEQ)**	**Sum of PCB 28, PCB52, PCB101, PCB138, PCB153 and PCB180 (ICES-6)[1]**
Meat, meat products and fat of bovine animals	2.5 pg/g fat	4.0 pg/g fat	40 ng/g fat
Liver of bovine animals	0.30 pg/g fresh weight	0.50 pg/g fresh weight	3.0 pg/g fresh weight

[1] Non-dioxin-like PCBs, or indicator PCBs are measured from a historical perspective. They are excluded from this risk assessment.
Please note According to footnote 33 of Regulation (EC) No 1881/2006, the level of fat for meat containing less than 2% fat must be calculated on a product basis!

The MLs for dioxins and dioxin-like PCBs in various foodstuffs are currently being updated at European level.

The Soil Quality Regulation[16] sets standards for soil concentrations of dioxins and dioxin-like PCBs in the Netherlands. This regulation has set the standards at 180 TEQ/kg soil (intervention value[17]) and 55 ng TEQ/kg soil (background value[18]) for the sum of dioxins and dioxin-like PCBs. However, these standards do not appear applicable to land in pastures and nature reserves (Hoogenboom et al., 2022b).

Hazard identification

Polychlorinated dibenzo-p-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) are collectively referred to as dioxins (EFSA, 2018a). These persistent substances are formed during incineration processes when the incinerated materials contain chlorine-containing components, e.g. PVC. Dioxins are also formed during various industrial processes. Polychlorinated biphenyls (PCBs) are highly stable and non-flammable substances, which for these qualities were mass-

[13] Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.
[14] As low as reasonably achievable, based on the distribution of residue levels found in the foodstuff.
[15] Commission Recommendation of 3 December 2013 on the reduction of the presence of dioxins, furans and PCBs in feed and food (2013/711/EU)
[16] Regulation of 13 December 2007, no. DJZ2007124397, laying down rules for the implementation of soil quality.
[17] A value that indicates a potentially serious reduction of soil's functional properties for humans, plants or animals, if exceeded.
[18] Defined as the level of good soil quality, for which there is no burden from local contamination sources.

produced in the past for various applications such as the use in transformers, paints, sealants and as heat exchange fluids.

Dioxins and dioxin-like PCBs attach in the environment to soil particles and sediment and are found everywhere in the environment. A distinction can be made between local contaminations, e.g. due to fires, industrial incidents, past industrial processes or old waste incinerators, and generic widespread contaminants.

In 2013, WFSR (then RIKILT) summarised and analysed the information available on the levels of dioxins in soil in relation to food safety (Hoogenboom & Traag, 2013). This analysis shows that the Lickebaert area between Vlaardingen and Maassluis and the areas around Zaandam are exemplary cases of local contaminations where a distinct correlation was found between emissions from a waste incinerator and high levels of dioxins in milk from cows from this area. Research carried out in these areas show that dioxins and dioxin-like PCBs are particularly found in the top layer of the soil, i.e. the top 10 cm (Hoogenboom & Traag, 2013). Also, the levels in the grass in winter and early spring were much higher than in summer. That said, soil levels were equally high in all seasons. Cattle grazing in the outdoors can be exposed to soil particles; the amount of soil depends on the grazing behaviour and varies among the different species (Hoogenboom & Traag, 2013).

Floodplains may be contaminated through disposal of river silt attached with contaminants. Limited data on floodplains showed that high levels of dioxins and dioxin-like PCBs have been found in these areas (Hoogenboom & Traag, 2013). In 2012, for example, high levels were measured in the grass and soil of the IJssel floodplains. The WFSR's analysis showed that little research had been carried out into the possible transfer to animal products from wild cattle grazing in floodplains. One exception is a study from 1996 on the relationship between levels of dioxins in the soil of three floodplains of Lek and elevated levels of dioxins in milk from cattle grazing in these areas (Hoogenboom & Traag, 2013). Although the milk showed elevated levels of dioxins, the congeneric pattern of milk and soil were different. It is unclear whether this is due to another source of contamination or a different transfer of cogeneration. Multiple studies on floodplains in Germany and the United Kingdom showed no clear link between levels of soil and the level in grass or animal products (Hoogenboom & Traag, 2013).

Hazard characterisation
Dioxins and dioxin-like PCBs form a group of different manifestations (congeners) that occur in different combinations but exhibit the same toxic profile. Mixture toxicity is therefore present. Because the potential of the various dioxins and dioxin-like PCBs differs for a toxic effect, Toxic Equivalency Factors (TEFs) have been developed for the congeners that accumulate in humans. The weighted levels can be added based on TEFs and determine the Toxic Equivalent (TEQ) of the mixture (EFSA, 2018a). This TEQ allows for the mixture to be assessed as one single substance.

Non-dioxin-like PCBs, or indicator PCBs, show a different toxicological profile than dioxins and dioxin-like PCBs (EFSA, 2005). For individual congeners and mixtures of non-dioxin-like PCBs, EFSA considers the toxicological data available insufficient to derive a health-based guidance value. The presence of a health-based guidance value is one of the factors needed for a hazard characterisation. As this is missing, BuRO cannot characterise the risk for the non-dioxin-like PCBs.

Male reproductive toxicity in boys and men is the critical toxicological effect of dioxins and dioxin-like PCBs (EFSA, 2018a). Infants aged 12 months have the highest exposure per kg body weight due to maternal exposure during pregnancy and breastfeeding. High exposure can have a detrimental effect on the infant's sperm quality in later life. Exposure is also associated with a delayed onset of puberty and a change in the male-female offspring ratio. Exposure to dioxins during pregnancy are also associated with higher plasma concentrations of the thyroid hormone TSH and dental problems in offspring.

At the end of 2018, EFSA established a Tolerable Weekly Intake (TWI)[19] of 2 pg WHO-TEQ/kg bodyweight/week (EFSA, 2018a). This TWI is based on studies in humans into the effects of dioxins and dioxin-like PCBs. Information from animal studies is considered to be supporting evidence. Determination of this TWI included exposure from breastfeeding and *in utero* exposure using kinetic models. EFSA considers the end of the breastfeeding period to be the critical time of exposure and has therefore used it to base the TWI on. This TWI is a factor seven lower than the TWI that had been derived in 2001 by the Scientific Committee on Food (SCF), namely 14 pg WHO-TEQ/kg body weight/week (SCF, 2001). EFSA has not derived a health-based guidance value for acute health effects of dioxins and dioxin-like PCBs.

Dioxins and dioxin-like PCBs accumulate in the body, particularly in body fat and liver whereby the levels in the liver are relatively higher due to so-called hepatic sequestration[20]. Dioxins and dioxin-like PCBs are excreted in milk and eggs (EFSA, 2018a).

Exposure estimate

<u>Concentrations of dioxins and dioxin-like PCBs in wilderness meat</u>

WFSR has conducted research in different Dutch floodplains on the level of dioxins and dioxin-like PCBs in kidney fat and liver of wild cattle grazing there year-round (Hoogenboom et al., 2022b). Kidney fat and livers of these bovine animals are not consumed. Kidney fat is a matrix that can also be sampled when animals are lean and therefore have low body fat. The level of dioxins in kidney fat is assumed to correspond with the level in meat fats, for which a risk assessment has been carried out (Hoogenboom et al., 2022b).
The initial research design described the sampling of as homogeneous a group of animals as possible. The preference was given to young bulls, as these animals were expected to have the highest levels of dioxins. However, the sampling depended on the accidental availability of animals, resulting in a more varied sampled group in terms of composition, both in sex and age.

In coordination with BuRO, the locations of the bovine animals studied by WFSR had been selected according to geographical location and the availability of animal tissue for the research. In the end, the animals sampled came from fourteen locations along the Waal (Beuningen and Loevestein), the Rhine (Millingen, Gendt, Ooijpolder, Loowaard, Meinerswijk, Blauwe Kamer, Lest, Prins Willem III plantation (PWIII) and Amerongen (Border Meuse and Koornwaard) and Merwede (Noordwaard). Depending on availability, Rode Geuzen or Galloways were sampled for the research. WFSR determined the levels of dioxins, non-like dioxin PCBs and non-dioxin-like PCBs.

[19] Tolerable Weekly Intake (TWI) is an estimate of the amount of a substance that can be ingested on a weekly basis over a lifetime without it having a noticeable effect on one's health.
[20] Hepatic sequestration (accumulation in the liver) is most likely caused by the binding of TCDD (and some other dioxins) to the liver enzyme cytochrome P450 1A2.

BuRO uses the data in the calculations below on the sum of dioxins and dioxin-like PCBs, given that the total TEQ levels of these substances are relevant for the assessment of health risks.

Levels of dioxins and dioxin-like PCBs in kidney fat

The results of the levels of the sum of dioxins and dioxin-like PCBs in kidney fat are summarised in Table 2. These data show that the levels in 40 out of 55 samples (73%) exceeded the ML. The majority of the samples that did not exceed the ML, or only in relation to very young animals, were found only in one area along the Meuse (Koornwaard) and three areas along the Rhine (Millingen, Gendt and Loowaard). The floodplain at Millingen has been reclaimed with sand, which may possibly explain the lower levels (Hoogenboom et al., 2022b). The levels in samples from Loowaard at the Rhine approximated the ML. The highest levels of dioxins were found in three animals from the floodplains at Loevestein that had been slaughtered after the winter period, thereby exceeding the ML up to a factor of 15. These levels were considerably higher than the other measurements. Based on two areas, the levels in animals from the floodplains along the Meuse were lower compared to areas along the Waal and the Rhine. However, the two animals from Border Meuse also slightly exceeded the ML.

Table 2: Measurement results of the WFSR research on kidney fat (Hoogenboom et al., 2022b). The table shows the levels for the sum of dioxins and dioxin-like PCBs.

Area	Number of animals	Number of animals exceeding ML[1]	Lowest level measured (pg TEQ/g fat)[1]	Highest level measured (pg TEQ/g fat)[1]
De Waal				
Beuningen (Gelderland)	3 cows	3	11.2	12.9
Loevestein[2]	5 bulls 1 cow	4	2.1	59.5
The Rhine				
Millingen	3 cows	0	2.7	3.6
Gendt	1 cow 1 bull	0	1.7	1.9
Ooijpolder	6 cows	5	4.7	19.7
Loowaard	3 cows 1 bull	1[3]	2.7	5.3
Meinerswijk	3 bulls	3	9.0	12.2
Blauwe Kamer	6 bulls	6	9.9	28.1
Elst (Utrecht)	3 cows	3	8.9	12.9
PWIII	3 cows	3	7.4	12.9
Amerongen	3 cows	3	5.5	10.8
The Meuse				
Border Meuse	2 bulls	2	4.7	5.5
Koornwaard	7 cows 2 bulls	3[4]	2.4	9.4
De Merwede				
Noordwaard	2 bulls	1	3.6	5.3
Total	55	40	-	-

[1] For the sum of dioxins and dioxin-like PCBs. The prevailing ML is 4 pg TEQ/g fat. A measurement uncertainty of 15% is taken into account in assessing the standard.
[2] At the Loevestein location, animals had been slaughtered in two seasons. In April 2021, three bulls measured extremely high levels, namely 54 – 59.5 pg TEQ/ g fat. In November 2021, the levels in two bulls and a cow were considerably lower, at 2.1 – 5.1 pg TEQ/ g fat.
[3] The ML excess was observed for the 3-year-old bull.
[4] The three animals that exceeded the ML were all under six months old when they were slaughtered.

Levels of dioxins and dioxin-like PCBs in livers, effects of sex and age**.**

WFSR also determined levels in liver in 42 of the animals tested. The results correspond to the findings for kidney fat: the ML was found to be exceeded in 30 out of 42 samples (71%). The levels in liver are not used for the risk assessment as operators no longer offer these bovine livers for consumption purposes. These results are therefore not discussed in detail.

Based on the measurement data available, WFSR concludes that the levels of dioxins measured in bulls' kidney fat and livers are a factor three higher than in cows (Hoogenboom et al., 2022b). This difference in sex is supported by the comparison of animals from two nearby floodplains: the levels of dioxins in Rode Geus bulls from Loevestein appear to be a factor four and a half higher than the Rode Geus cows from Beuningen, while between these two areas along the Waal the levels in soil and grass are fairly similar. This difference between the sexes may possibly be explained by lower fat content of bulls, and thus a stronger concentration of dioxins, especially in winter time, and the fact that cows excrete dioxins through lactation. WFSR also concludes that young animals were observed to have relatively higher levels of dioxins compared with older animals (Hoogenboom et al., 2022b). All cattle from Koornwaard in which the ML was exceeded had been slaughtered at an age of less than one year. The relatively high level of dioxins in these animals may be due to a high exposure to milk during the animals' first phase of life. Growth dilution causes these levels to decrease later in life. Also, the animals that had measured the highest levels, i.e. three bulls from the Loevestein area, were still relatively young, at about three years of age.

Comparison of the level of dioxins in kidney fat with muscle fat of fatty meat and lean meat

The measurements in the WFSR research were performed on kidney fat as this is easier to sample in this matrix. In order to verify whether the level of dioxins in kidney fat corresponds with the level of dioxins in meat fat, WFSR measured the levels of dioxins in liver fat, lean meat and fatty meat for the three animals from the Loevestein area (Hoogenboom et al., 2022b). This comparison shows that the levels of dioxins in liver fat are a factor three to four higher than in kidney fat. This is in line with the accumulation of dioxins in the liver due to hepatic sequestration. After a correction for the fat percentage, the levels of dioxins in kidney fat appear to correlate well with the level in fat meat for meat with a fat percentage of more than 1.5%. Based on a limited number of measurements, the level of dioxins in kidney fat in lower fat percentages appears to be a factor two to three higher than the level of dioxins in meat fat. It may therefore be possible that the assumption that kidney fat correlates with meat fat leads to an overestimation of the concentration of dioxins and dioxin-like PCBs in whole lean meat. At present, there are insufficient data to be able to determine the exact distribution of these substances across the fat compartments.

Levels of dioxins and dioxin-like PCBs in the floodplains' soil

WFSR also determined levels of dioxins and dioxin-like PCBs in soil at the grazing areas of the cattle sampled (Hoogenboom et al., 2022b). The background values determined in this research for areas outside the Dutch floodplains are in the same order of magnitude for all areas: between 0.97 and 1.52 pg TEQ/kg DM. For the Beuningen and Ooijpolder areas, a comparison was made between levels of dioxins in the soil of the floodplain and levels of dioxins in the soil on the other (dry) side of the embankment. This comparison showed that the level of dioxins was significantly higher in the soil of the floodplain. The floodplain soil levels of Beuningen area were measured between 10.96 and 18.57 pg TEQ/kg DM for the sum of dioxins and dioxin-like PCBs. Conversely, the level at the other side of the embankment was 1.52 pg TEQ/kg DM. Also, the Ooijpolder area showed a difference between the levels of soil on both sides of the embankment of more than a factor of fourteen, whereby the level of dioxins on the dry side of the embankment was at 0.97 pg TEQ/kg dry DM. The levels of dioxins outside the floodplain matched those of the soil of a monitoring area, a 'clean' farm in Elst, Gelderland: 1.21 pg TEQ/kg DM (Hoogenboom et al., 2022b).

The research measured soil levels significantly above background values in all floodplains where animals were sampled. The floodplains of Millingen (reclaimed with sand), Koornwaard along the Meuse and Border Meuse proved an exception to the above. A least for Millingen and Koornwaard, these lower soil levels also translated themselves into lower levels in the animals' kidney fat and liver (Hoogenboom et al., 2022b).

The soil measurements show that the soil levels in one floodplain can strongly differ within one flood area. For example, in the floodplains of Meinerswijk, levels were measured between 9.51 and 70.66 pg TEQ/kg DM (Hoogenboom et al., 2022b). Possible explanations for these differences are found in flood frequency or the presence of a historical source of contamination.
Measurements before and after flooding of the floodplain near Beuningen showed limited effect of flooding on soil levels (Hoogenboom et al., 2022b). With the

exception of two measurements, the soil levels remained below the background value[21] of 55 pf TEQ/kg ground as laid down in the Soil Decree. However, previous calculations of WFSR show that it requires lower levels of soil to not exceed the ML in animal products (Hoogenboom et al., 2022b).

Levels of dioxins and dioxin-like PCBs in grass

WFSR determined the levels of dioxins in grass in six Dutch floodplains and one monitoring area (a farm in Elst, Gelderland) (Hoogenboom et al., 2022b). The levels in the floodplains' grass turned out to be a factor two higher than the levels in areas outside the floodplains, though in most cases they did meet the ML for feed of plant origin, i.e. 1.25 ng TEQ/kg for the sum of dioxins and dioxin-like PCBs.

The levels of dioxins and dioxin-like PCBs in grass were temporarily greatly elevated following a flood, presumably due to deposition of contaminated silt. Measurements of grass from one and the same place in the floodplain at Beuningen showed that the level is at 0.04 ng TEQ/kg without floods. After flooding, this level could increase up to 1.2 ng TEQ/kg after a flood in July 2021 followed by heavy rains, and 8.54 ng TEQ/kg following a flood in February 2021.

Transfer of dioxins and dioxin-like PCBs from soil and grass to cattle

Development and validation of transfer models

RIVM and WFSR have developed models that estimate the transfer of dioxins and dioxin-like PCBs from soil and grass to the Rode Geus' meat fat and liver (Minnema et al., 2021). Three models have been developed to describe the different animals of a cattle: one for adult non-lactating bovine animals, mature lactating cows and growing calves less than one year old. These models are based on previously developed and optimised RIVM models and existing data on the intake of grass and soil by regular bovine breeds. The models take seasonal factors on body weight into consideration (the amount of fat) as well as the growth of calves and the levels in grass. The models can also simulate the decrease of levels in cattle when rehomed to a clean area. A clean area is understood to be a location free from additional intake of dioxins.

The models have used levels of dioxins and dioxin-like PCBs in soil and grass, expressed in TEQ, that have been measured by WFSR in the floodplains at Beuningen (Minnema et al., 2021; Notenboom et al., 2021; Hoogenboom et al., 2022b). The data from Beuningen are applied because most of the data are available for this area, and because, given its central location along the Waal, it is considered a representative area of the Waal floodplain.
The models describe a realistic scenario and a worst-case scenario (Minnema et al., 2021). Elevated levels of dioxins in grass were used in the worst-case scenario, which were measured directly after a flood in Beuningen due to deposition of contaminated silt. The realistic scenario made use of lower, more common levels of dioxins in grass. The soil levels are the same in both scenarios. Intake of dioxins and dioxin-like PCBs by a calf through milk consumption was modelled for the first six months for both scenarios with the average TEQ concentration in milk estimated with the lactating cow transfer model. It is assumed that calves aged six months to a year are only exposed to dioxins through the intake of grass and soil. This exposure is determined on the basis of grass and soil samples taken outside the Beuningen floodplain.

[21] Defined as the level of good soil quality, for which there is no burden from local contamination sources.

Validation and applicability of the transfer models
A first validation of the RIVM model made use of the levels of dioxins and dioxin-like PCBs measured by WFSR in the liver and kidney fat of three lactating cows from the floodplains at Beuningen (Minnema et al., 2021). These levels of dioxins are measured at or above the simulated levels of dioxins assuming a worst-case scenario. This validation made use of the levels of dioxins measured by WFSR in kidney fat for the simulation of meat fat. From these validations, BuRO concludes that the model requires further fine-tuning before it can actually be put into practice for a quantitative description of transfer. At this stage, the models can mainly be applied to simulate variations and trends throughout the year. RIVM and WFSR also make recommendations for the model's further fine-tuning and validation (Notenboom et al., 2021)**.**

Simulations of levels in meat fat for the Beuningen area
RIVM used the transfer models, based on the available levels of dioxins in soil and grass, to simulate the levels of dioxins in fat and liver of non-lactating cattle, lactating cows and calves in the floodplain near Beuningen (Notenboom et al., 2021). RIVM simulated progression over a four-year period for adult non-lactating cattle and lactating cows. The effect of rehoming to a clean stable or pasture (no additional dioxin intake) was simulated as of the month of April in the third year, with the highest level of dioxins in meat fat. By starting from the highest level, RIVM simulated the reduction under worst-case starting conditions. A one-year simulation was carried out for the growing calf model, which assumed exposure from milk after six months, and a background exposure via soil and grass for the second six months.

The simulations show that the level of dioxins and dioxin-like PCBs in meat fat of calves, adult non-lactating cattle and lactating cows exceed the ML of 4 pg TEQ/g fat in both the realistic and worst-case scenario (Table 3) (Notenboom et al., 2021). Assuming a realistic scenario, the levels of dioxins in meat fat of lactating cows temporarily drop below the ML during the lactation period because of the excretion of dioxins and dioxin-like PCBs in milk. For the calves, however, this is exactly the period in which the levels of dioxins in meat fat are highest. The level of dioxins in meat fat has a variation over time on account of seasonal factors: the lowest levels are reached in late summer and early fall. Except for lactating cows, these lowest levels exceed the ML in the realistic scenario. BuRO notes that any growth dilution in the models for adult animals is not yet included in the model; in young adulthood, animals still grow.

Table 3: Simulated levels of dioxins in bovine meat fat from the Beuningen floodplain and the period needed to arrive at a level of dioxins below the ML after the cattle had been rehomed to a clean stable or pasture (Notenboom et al., 2021). RIVM set the sampling period from the highest simulated level of dioxins. Levels are the sum of dioxins and dioxin-like PCBs expressed in pg TEQ/g fat.

Model	**Realistic scenario**			**Worst-case scenario**		
	Lowest level according to simulation	**Highest level according to simulation**	**Sampling period up to below ML**	**Lowest level according to simulation**	**Highest level according to simulation**	**Sampling period up to below ML**
Calf	-	8.8	> 6 months[1]	-	26.8	> 6 months[1]
non-lactating cattle	4.4	7.7	37 days	7.0	32	110 days
Lactating cow	3.3	7.7	24 days	4.5	32	76 days

[1] For calves, the period of only background exposure has been simulated for a period of six months.

The moment the intake of dioxins through consumption of grass and/or soil from the floodplain is equated to the background exposure, the levels of dioxins in meat fat decrease for all models. From the highest simulated level, in a realistic scenario, it takes 37 days, 24 days and more than six months respectively before the level of dioxins reaches below the ML for adult non-lactating cattle, lactating cows and calves. In a worst-case scenario, this period is longer for non-lactating cattle and lactating cows: 110 days and 76 days respectively. BuRO notes that any growth dilution has not been included in the models for adult cattle.
The models also simulate the levels of dioxins in bovine liver after the consumption of grass and/or soil from the flood plain. According to the worst-case scenario, the total level of dioxins in the liver exceeds the ML of 0.5 pg TEQ/g fresh weight (Notenboom et al., 2021). For calves, the ML is exceeded in both the worst-case scenario and the realistic scenario.

Seasonal factors on levels of dioxins and reductions following relocation to a clean environment

Cattle from the Loevestein area were slaughtered in three periods: June 2020 (summer, before the start of the WFSR research; these data do not form part of the current data set), April 2021 (after the winter period) and in November 2021 (Hoogenboom et al., 2022b). The levels of dioxins in kidney fat were substantially higher after the winter period, at 54.0 to 59.5 pg TEQ/g fat, than in June, at 7.6 to 28.9 pg TEQ/g fat, and November, at 2.1 to 5.1 pg TEQ/g fat. Given the large difference in levels in cattle from the same area, this finding presents an indication of seasonal factors, possibly caused by loss of fat in the winter period (Hoogenboom et al., 2022b).

To explore a possible line of action, in the event that bovine animals have excessive levels of dioxins, WFSR conducted research on the effect of rehoming them to a clean environment (lowering exposure) on levels of dioxins. This was done by examining the levels in blood plasma and kidney fat (Hoogenboom et al., 2022a). As no animals need to be slaughtered for the collection of blood samples, different samples can be taken over time. This enables the monitoring of the time progression of the levels of dioxins. Kidney fat can only be collected by slaughtering the animals. Two operators took initiatives to rehome cattle and made samples available to WFSR. The effect of the relocation was examined in four groups of animals as follows (Hoogenboom et al., 2022a).

1. Five Rode Geus bulls from the floodplain near Loevestein were stabled. In the first two months, the blood levels of dioxins, dioxin-like PCBs, the sum of dioxins and dioxin-like PCBs and non-dioxin-like PCBs reduced by 92%. Five months later, the blood levels proved to be 97% lower than in the initial situation, which corresponds to the measurement in kidney fat after the slaughter. The animals therefore met the MLs for the fat contents. According to WFSR, the decrease in levels is mainly explained by the reduction of exposure and by growth and fattening of these animals and, to a small extent, by excretion.

2. The relocation of four oxen of different breeds of cattle from De Bakenhof floodplain near Arnhem to a clean farm in Elst, Gelderland, showed a drop in blood levels of 56% in the first three months for the sum of dioxins and dioxin-like PCBs. This drop did not appear to continue four months later; in fact, there was an increase. After four months, the animals rehomed to a different pasture in an area with slightly higher levels of soil. However, the measured soil levels cannot fully explain the increase according to WFSR, which means that possible other factors, such as weight loss, also played a role.

3. Five of eight Rode Geuzen from the Gendtse waard were rehomed to a pasture outside the floodplain near Ooij. The levels in blood plasma of these five animals appeared to be higher at slaughter than those of the animals that had not been rehomed. The relocation thus turned out to have an adverse effect. This observation does not correspond with the levels measured in the soil and grass of the two areas.

4. The blood plasma of eight Scottish Highland bulls were compared to examine the effect of the relocation. Two animals were from Broekpolder, which is a polder with high soil levels, no floodplain, and were not relocated. Three animals were rehomed from Broekpolder to a clean area in Bieschbosch that does not flood called Lijnoorden and three animals were relocated from Noordwaard to Lijnoorden. The levels in kidney fat of the relocated animals were compared to the kidney fat of the cattle that had not been moved. The bovine animals in Lijnoorden were supplemented with hay. The levels of dioxins in these animals decreased by 25% to 47% for the sum of dioxins and dioxin-like PCBs. By contrast, the levels of non-like-dioxin PCBs slightly increased; the cause of this is unknown.

Based on the above findings, BuRO concludes that rehoming cattle to a clean environment and thus reducing the exposure could lead to a reduction of levels of dioxins. This particularly applies to animals still growing or putting on fat. Except in lactation, excretion plays a lesser role. Stabling these animals appears to be the most effective approach, though this may have consequences for animal welfare given that these cattle breeds are particularly adapted to frequent outdoor grazing. BuRO has not carried out any research into the practical feasibility of such a measure and the effects they may have on animal welfare. In a number of cases, it appears that those animals rehomed to a clean pasture are still exposed to dioxins and dioxin-like PCBs. When rehoming cattle to another area, it is thus vital to properly map soil levels in the area beforehand in order to minimise exposure from the soil.

Offer and consumption of wilderness meat

To get a better focus on the consumption of wilderness meat, BuRO requested data from FO on meat marketing in the Netherlands, which meat products are being sold to consumers and how often consumers eat this meat. Wilderness meat serves a specific consumer market segment and has a modest volume.

FO obtained the information from three foundations that offer wilderness meat (Front office, 2021). According to this analysis, the total marketing of wilderness meat is a maximum of 70,000 kg per year and this meat is from animals from floodplains and other nature reserves. The foundations estimate that wilderness meat is sold to approximately 4,000 private customers, mostly families regularly eating this meat. Upon enquiry, FO estimates that approximately one third of the meat is sold via regular channels, not labelled as wilderness meat. This meat is not eaten by a specific group and the number of customers is not known.

The wild cattle is slaughtered all year long, though less often in the period between March and June. Wilderness meat is sold year-round and throughout the Netherlands (Front office, 2021). Wilderness meat is sold via the internet, as deep frozen, bundled packages and offered to consumers at specific pick-up points. These packages consist of meat and meat products such as minced meat, burgers, sausages and steak tartare. The packages range in quantities from 3 to 8 kg. Most of the meat is lean (≤2% fat) and the meat products vary from 10% (chopped steak), 25% (minced meat) to 30% (sausages). Offal and fat are no longer being offered by the operators. The meat in one package comes from multiple animals originating from the same area.

Wilderness meat is presumably eaten by all different age groups. According to the foundations, there are consumers who eat this meat twice a week. The Free Nature foundation estimates that consumers eat 100 to 150 grams of meat at a time. That would correspond with a maximum consumption of 300 grams a week. Based on the Dutch Food Consumption Survey[22], the estimated consumption quantities of regular beef for adults on days when beef is eaten are around 85.6 grams per day on average and a P95 of 200 grams per day. However, the estimated frequency of eating wilderness meat (twice a week) is slightly higher than the frequency of regular beef consumption according to the food consumption survey, which is namely 16% of the days, or 1.12 times a week.

BuRO calculates the average consumption of wilderness meat on the basis of the total supply and the number of customers, as reported by FO (Front office, 2021), in the following manner. The supply is 70,000 kg a year, of which about two thirds is brought to market as wilderness meat. The total is thus 47,000 kg of wilderness meat. This 47,000 kg of wilderness meat is then sold to approximately 4,000 private customers, which corresponds to approximately 12 kg per customer per year.

BuRO assumes that a customer usually consists of a family of 2.14[23] persons, as indicated in the FO analysis. The consumption of wilderness meat then comes down to 5.6 kg per person per year, assuming that everyone in the family eats equal amounts and no meat is wasted, in other words about 110 grams a week. For large eaters of this meat, BuRO keeps the highest estimate in the FO report: 300 grams per week (Front office, 2021).

Intake calculations for dioxins and dioxin-like PCBs

Multiple factors depend on the intake calculation for dioxins and dioxin-like PCBs by the consumption of wilderness meat: the levels of dioxins and dioxin-like PCBs in fat, the fat content of the meat, the amount of meat being eating and the frequency at which this meat is being eaten.

When calculating the intake for chronic exposure, BuRO assumes a P50 level (median or 50th percentile of concentration distribution) of dioxins and dioxin-like PCBs in fat. BuRO based this value on WFSR's 55 measurement results in kidney fat. This approach provides a representative overview of the levels of dioxins in wilderness meat as the meat packages are sold throughout the Netherlands and therefore consumers who eat this meat regularly will buy meat from different areas in the Netherlands (Front office, 2021). By using these levels of dioxins, BuRO assumes that all wilderness meat comes from floodplains, however, wilderness meat can also originate from other nature reserves where flooding with contaminated silt cannot occur. The calculated intake of dioxins through the consumption of wilderness meat could therefore be an overestimation.

Determining the P50 and P95 levels in kidney fat

Based on a Jarque-Bera test of normality, with a P-value of 0.05, BuRO concludes that the data on concentration (55 data points) from the WFSR study are not normally distributed. The P50 and P95 are therefore determined after a logarithmic transformation of the dataset.

[22] https://statline.rivm.nl/
[23] https://www.cbs.nl/nl-nl/visualisaties/dashboard-bevolking/woonsituatie/huishoudens-nu (consulted on 8 December 2021)

Based on the entire dataset, BuRO sets the P50 at 7.1 pg TEQ/g fat and the P95 at 34 pg TEQ/g fat. The three samples taken from the Loevestein area in the winter period, which measured extremely high levels, strongly impacted the P95. After exclusion of these measurement points, the P50 and the P95 are 6.2 and 14.2 pg TEQ/g fat respectively. BuRO uses the P50 level of the entire dataset (7.1 pg TEQ/g fat) for the intake calculations as this is the most representative value for the chronic intake calculations. It is unlikely that a consumer eats large quantities of wilderness meat with P95 levels for a long period of time.
In its intake calculations, BuRO assumes that the measured level of dioxins in kidney fat corresponds to the level of dioxins in meat fat. However, the comparison of WFSR shows that the use of kidney fat levels may result in an overestimation of the levels of dioxins in lean meat with a fat percentage of less than 1.5% (Hoogenboom et al., 2022b).

Comparison of the levels of dioxins in wilderness meat and regular[24] beef

WFSR supplied a dataset with measurements of dioxins of 109 samples of regular beef, measured under the National Plan spanning the period 2013 to 2020. Based on the sum of dioxins and dioxin-like PCBs, one sample exceeds the ML by 0.9%: 5.16 pg TEQ/g fat. The levels measured are in the range of 0.16-5.16 pg TEQ/g fat, the P50 is 0.18 pg TEQ/g fat and the P95 (95th percentile of the concentration distribution) is 0.37 pg TEQ/g fat. The levels in regular beef are therefore well below the levels found in wilderness meat. See table 4.

[24] Cattle kept as farm animals.

Table 4: The P50 and P95 levels of dioxins and dioxin-like PCBs calculated by BuRO.

	P50 level (pg TEQ/g fat)	**P95 level (pg TEQ/g fat)**
Wilderness meat	7.1	34
Regular beef	0.18	0.37

Weekly intakes of dioxins and dioxin-like PCBs from wilderness meat by adults

Based on the FO assessment (Front office, 2021) on the consumption of wilderness meat, BuRO assumes that a wilderness meat consumer eats between 110 and 300 grams of wilderness meat per week, 110 grams being the average target group consumption. According to the FO assessment, the meat usually has a 2% fat content. BuRO considers the fat percentages in meat products as estimated by FO (10 to 30%) to be high. That is why BuRO calculated the average fat percentage of the meat products in the package based on the composition of the meat packages[25] and the data on regular beef from the Dutch Food Composition Database[26] (NEVO). In so doing, BuRO works on the basis of a fat percentage of 14%. The calculations are provided in Appendix 2.

Based on the above data, BuRO calculates four intake scenarios for adults:

- Scenario 1: consumers of wilderness meat eat 300 grams of meat per week (large quantity) with an average fat content of 14%.
- Scenario 2: consumers of wilderness meat eat 300 grams of meat per week (large quantity) with a low fat content of 2%.
- Scenario 3: consumers of wilderness meat eat 110 grams of meat per week (average quantity) with an average fat content of 14%.
- Scenario 4: consumers of wilderness meat eat 110 grams of meat per week (average quantity) with a low fat content of 2%.

These intake scenarios do not take any exposure from other sources into account. Four these four scenarios, BuRO calculates the total weekly intake from wilderness meat for levels of dioxins and dioxin-like PCBs equal to P50. See table 5. This is done based on the following formula:

$$\frac{(consumption\ amount\ x\ fraction\ of\ fat\ x\ total\ TEQ\ content\ in\ fat)}{body\ weight}$$

Depending on the scenario, the individual components of the formula are filled in as follows:

- Consumption amount: 110 g/week or 300 g/week
- Fraction of fat: 2% or 14%
- Total TEQ content in fat: 7.1 pg TEQ/g fat (P50).
- Body weight: 60 kg

[25] https://www.freenature.nl/wildernisvlees/kwaliteit-en-eisen/vleespakket-wat-zit-erin (consulted on 5 January 2022.)

[26] https://nevo-online.rivm.nl/

Table 5: Overview of the weekly intake of dioxins and dioxin-like PCBs from wilderness meat for a 60-kg adult based on the different intake scenarios.

Scenario	Meat consumption (g/week)	Fat percentage of meat (%)	Concentrations of dioxins and dioxin-like PCBs (pg TEQ/g fat)	Intake (pg TEQ/week)	Intake per kg of body weight
1	300	14	7.1	298.2	5.0
2		2		42.6	0.7
3	110	14		109.3	1.8
4		2		15.6	0.3

Weekly intakes of dioxins and dioxin-like PCBs in four intake scenarios, based on regular beef consumption

In order to calculate the additional intake of dioxins and dioxin-like PCBs from wilderness meat relative to regular beef, the four intake scenarios are calculated with the levels as these are found in regular beef. To do so, the levels used by WFSR are measured under the National Plan in the period between 2013 and 2020: a P50 of 0.18 pg TEQ/g fat and a P95 of 0.37 (pg TEQ/g fat). Table 6 summarises the intake from regular beef.

Table 6: Overview of the weekly intake of dioxins and dioxin-like PCBs from regular beef for an adult based on the different intake scenarios. P50 and P95 levels are based on measurements under the National Plan spanning the period from 2013 to 2020.

Scenario	Meat consumption (g/week)	Fat percentage of meat (%)	Intake (pg TEQ/week)	
			P50 level	**P95 level**
1	300	14	7.6	15.6
2		2	1.1	2.2
3	110	14	2.8	5.7
4		2	0.4	0.8

Comparison of intake from wilderness meat (Table 5) and intake from regular beef (Table 6) shows that the intake of dioxins and dioxin-like PCBs from wilderness meat is nearly a factor 40 higher.

Weekly intakes of dioxins and dioxin-like PCBs from wilderness meat by children

FO concludes that children are also likely to eat wilderness meat (Front office, 2021). The consumption amounts are unknown however, which is why BuRO calculates the amount of wilderness meat which a child of 12 kg can eat on a weekly basis before exceeding the TWI. BuRO then checks whether this scenario is realistic and whether the presence of dioxins and dioxin-like PCBs in wilderness meat can lead to health risks. This does not take account of intake from other sources. Children with a body weight of 12 kg may ingest 24 pg TEQ per week (2 pg TEQ/kg body weight/week x 12 kg) before the TWI is exceeded. The weekly amount of meat causing to exceed the TWI is calculated according to the following formula:

$$\left(\frac{24\ pg\ TEQ}{total\ TEQ\ content\ in\ fat}\right) \times \left(\frac{1}{fraction\ of\ fat}\right)$$

Depending on the scenario, the individual components of the formula are filled in as follows:

- Total TEQ content in fat: 7.1 pg TEQ/g fat (P50)
- Fraction of fat: 2% or 14%

Table 7 gives an overview of the calculated weekly consumption amounts whereby the TWI is exceeded.

Table 7: Overview of the weekly consumption amounts of wilderness meat for a child of 12 kg whereby the TWI is exceeded, at different fat percentages and levels of dioxins.

Fat percentage of meat (%)	Concentrations of dioxins and dioxin-like PCBs (pg TEQ/g fat)	Weekly consumption amount (grams)
14	7.1	24
2		169

Risk characterisation

In a risk characterisation, the exposure estimate is compared to the health-based guidance value. In this case, the health-based guidance value is the TWI of 2 pg TEQ/kg body weight/week as established by EFSA (EFSA, 2018a). For an adult person of 60 kg, the tolerable intake of dioxins and dioxin-like PCBs therefore comes down to 120 pg TEQ per week. Table 8 presents the ratio of the TWI and the calculated intake for an adult person for the different intake scenarios. A ratio of more than 1 exceeds the health-based guidance value, which means that a health risk cannot be ruled out. The intake from other sources has not been included in this approach. The starting point of the risk assessment is that wilderness meat that does not comply with the MLs would still be brought to market, which is not permitted.

Table 8: The ratio of the TWI and the weekly intake of dioxins and dioxin-like PCBs from wilderness meat with a P50 level, for an adult person of 60 kg.

Scenario	Meat consumption (g/week)	Fat percentage of meat (%)	Ratio of the TWI and the weekly intake
1	300	14	2.5
2		2	0.4
3	110	14	0.9
4		2	0.1

Intake at both medium and high consumption of lean wilderness meat (2% fat) does not exceed the TWI of dioxins and dioxin-like PCBs. The intake of average amounts of 110 g/week of wilderness meat products (14%) does not exceed the TWI either. However, a high consumption of 300 g/week of wilderness meat with 14% fat does exceed the TWI of dioxins and dioxin-like PCBs. This does not take account of intake from other foodstuffs.

If the intake scenarios are calculated with the levels of dioxins and dioxin-like PCBs that are measured in regular beef, this does not exceed the TWI.

A weekly consumption of 24 grams of meat products with 14% fat by children with a body weight of 12 kg (toddlers) exceeds the TWI. Lean meat (≤2% fat) requires an amount of 169 g/week before exceeding the TWI. According to RIVM's Dutch National Food Consumption Survey, the average daily beef consumption of one to three year-olds is 3.3 grams a day and the P95 consumption is 21 grams a day[27]. It is thus not likely that a child would eat more than 147 grams of

[27] https://statline.rivm.nl/

wilderness meat per week (P95 consumption amount of beef per week). Lean wilderness meat consumption by children will therefore not lead to health risks. Consumption of meat products with 14% fat exceeds the TWI at realistic consumption amounts. A health risk cannot therefore be ruled out.

Intake from the total diet

RIVM determined the total exposure to dioxins and dioxin-like PCBs from the total diet for the general Dutch population (Boon et al., 2014). The calculations were made using levels of dioxins based on Dutch monitoring data in the 2010-2013 period. The calculated exposure from the total diet was as follows.

- For children aged 2 to 6 the median intake is between 0.8 and 1 pg TEQ/kg body weight/day and the 95th percentile between 1.2 and 1.6 pg TEQ/kg body weight/day. The latter corresponds to 8.4 and 11.2 pg TEQ/kg body weight/day.
- For children aged 7 and above and adults (age group 7 to 69), the median intake is at 0.5 pg TEQ/kg body weight/day and the 95th percentile at 1.0 pg TEQ/kg of body weight per day. This corresponds to 3.5 and 7.0 pg TEQ/kg body weight/day.

The exposure from the total diet therefore exceeds the TWI set by EFSA for children and adults of 2 pg/kg body weight per week (EFSA, 2018a).

The calculations by RIVM show that, in addition to fish, milk and vegetable oils, meat, and beef in particular, form a significant source of dioxin intake. Beef contributes 14-16% of total intake (Boon et al., 2014).
If beef with elevated levels of dioxins is consumed on a regular basis, as is the case with wilderness meat, it will trigger an increase in the TWI overrun.

Conclusion

Levels of dioxins and dioxin-like PCBs in liver and kidney fat of wild cattle grazing in the floodplains year-round regularly exceed the ML. This is in contrast to measurements in regular beef, which measures much lower levels. The high levels in wilderness meat can be explained by the higher levels in the soil and grass of the floodplains and the possible longer exposure time of these animals. On four out of thirteen areas, the meat of the animals met or partly met the ML, possibly because of lower soil levels in those areas. The higher levels in soil and grass appear to be a more generic problem for floodplains. Measurements carried out by WFSR show an increase in dioxins and dioxin-like PCBs directly after floods, possibly caused by deposition of contaminated river silt. The highest levels are found in young animals and animals of the male sex. Possible contributing factors are the high exposure of calves through milk and lower fat content of the animals which concentrates dioxins.

BuRO's risk assessment shows that regular consumption of the wilderness meat products with an average fat content of 14% leads to an excessive intake of dioxins and dioxin-like PCBs in case of high consumption (300 g/week). The TWI is not exceeded at an average consumption of 110 g/week, however, there is little room left for safe intake of dioxins and dioxin-like PCBs from other sources. For children of 12 kg, the consumption of realistic amounts of wilderness meat results in the TWI being exceeded. Consuming lean meat (≤2% fat) does not exceed the TWI for both children and adults. If the levels from regular beef are kept, the TWI is not exceeded in any of the scenarios and the intake of dioxins and dioxin-like PCBs is almost a factor 40 lower. Exposure to dioxins and dioxin-like PCBs from the total Dutch diet is too high and compared with regular beef, wilderness meat consumption may thus add to the total intake. This line of reasoning assumes that consumers of wilderness meat otherwise have the same diet as consumers of regular beef.

Both the simulations with the transfer model and the limited number of measurements in kidney fat show variations in the levels of dioxins and dioxin-like PCBs owing to seasonal factors. Simulations and measurements also show that the levels in kidney fat can drop to below the ML after the (growing) animals have been rehomed to a clean environment. In this context, however, it is important that these locations are not affected by local contaminations. BuRO has not carried out any research into the practical feasibility of such measures and the effects they may have on animal welfare.

PART 2: HEAVY METALS

Legal framework

Regulation (EC) No. 1881/2006[28] sets out the European MLs for the presence of cadmium and lead in beef and bovine organs. Regulation (EC) No. 396/2005[29] sets European maximum residue levels for mercury and copper as residues of pesticides. However, no distinction can be made in the source of these contaminants. The MLs are summarised in Table 9. There are no MLs for nickel and arsenic for meat.

Table 9: MLs of heavy metals in bovine meat, liver and kidneys according to Regulation (EC) No 1881/2006 and Regulation (EC) No 396/2005.

Description	Cadmium (mg/kg)	Lead (mg/kg)	Mercury (mg/kg)	Copper (mg/kg)
Meat of bovine animals	0.050	0.1	0.01	5
Liver of bovine animals	0.5	-	0.02	30
Kidney of bovine animals	1	-	0.02	30
Slaughterhouse waste	-	0.2[1]	-	-

[1] The ML for lead in slaughterhouse waste from bovine animals was reduced from 0.5 to 0.2 mg/kg from 1 January 2022. The higher ML was thus still in force at the time of the study.

BuRO's research in 2008 shows that the cadmium levels in bovine kidneys increases proportionally with the animals' age (BuRO, 2008). This is why the kidneys of bovine animals older than two years are declared unfit for consumption purposes in the Netherlands (NVWA, 2019).

Hazard identification

Heavy metals naturally occur in soil. In addition, heavy metals may be present as contamination as a result of deposition of contaminated silt or as a result of regional atmospheric deposition from industrial activities. The use of leaded petrol, for example, was a major source of environmental lead contamination in the past. Lead, cadmium, mercury, copper and nickel form part of the heavy metals. Metalloid arsenic has also been analysed.

Copper is an essential trace element; both deficiency and excess intake of a trace element can be detrimental to health. This risk assessment only assesses the effects of excessive intakes. Copper is naturally present in plants. In addition, there are also a number of copper-containing pesticides and copper is added to animal feed to prevent deficiencies. Nickel is a trace element and naturally present, or present everywhere in soil, water and air as a result of human action. Nickel is most commonly found in food and drinking water as nickel(II).

Hazard characterisation

Cadmium accumulates in kidneys and livers and may cause kidney failure (EFSA, 2011). Cadmium is also associated with increased risk of developing cancer. EFSA has established a TWI for cadmium at 2.5 µg/kg body weight per week based on renal toxicity.

[28] Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.
[29] Regulation (EC) No. 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EC.

Lead can accumulate in the human body and cause damage to the developing nervous system. Lead furthermore causes high blood pressure and renal toxicity in adults. EFSA has derived lower confidence limits of the benchmark dose values (BMDL) for these effects for the purpose of risk assessment: a $BMDL_{01}$[30] of 0.5 µg/kg body weight per day for toxicity to the developing nervous system, a $BMDL_{01}$ of 1.5 body weight per day for cardiovascular effects and a $BMDL_{10}$[31] of 0.63 µg/kg body weight per day for renal toxicity, used for adults as developmental toxicity is no longer a factor for them (EFSA, 2010).

There are two types of arsenic, organic arsenic and inorganic arsenic. Inorganic arsenic is especially highly toxic. The organic types are believed not to lead to adverse effects in very small (micromolar) amounts. There is no specific information available on the toxicity of organic arsenic compounds at higher amounts to make a statement on toxicity (EFSA, 2009). Inorganic arsenic may cause bladder, lung, and skin cancer and skin lesions. Based on the aforementioned health effects, EFSA's CONTAM panel has established a lower confidence limit of the benchmark dose ($BMDL_{01}$) of 0.3 to 8 µg/kg body weight per day for inorganic arsenic (EFSA, 2009). Joint FAO/WHO Expert Committee on Food Additives (JECFA) has derived a $BMDL_{0,5}$[32] of 3 µg inorganic arsenic/kg body weight per day (range of 2.0-7.0 µg/kg body weight per day) for increased risk of lung cancer (JECFA, 2011). ECHA has selected a $BMDL_{0,5}$ for cancer of 3.0 µg inorganic arsenic/kg body weight per day (ECHA, 2013). The application of a factor 10 (Margin of Exposure) on a $BMDL_{0,5}$ of 3 µg/kg body weight per day leads to 0.3 µg/kg body weight per day (BuRO, 2021).

Long-term exposure to methylmercury can lead to adverse effects on neurodevelopment. Methylmercury is the most toxic and also most common type of mercury in food and is mostly found in fish (EFSA, 2012; Boon et al., 2017). EFSA has established a TWI for methylmercury at 1.3 µg/kg body weight per week, expressed as mercury.

Copper is a trace element involved in the formation of connective tissue and bones. Acute toxic effects of copper result in local gastrointestinal irritation. Chronic exposure to copper can result in liver injury (EFSA, 2015). For pesticides containing copper, EFSA has derived an ADI of 0.15 mg/kg body weight per day (EFSA, 2018b). Copper is also an essential trace element for which EFSA has set an upper level (UL) of 5 mg/day for adults and 1 mg/day for children aged one to four (EFSA, 2006; 2015). Coordination of both values is still ongoing at EFSA (EFSA, 2021).

The critical effect for acute oral exposure to nickel are eczematous flare-up reactions in the skin elicited in nickel-sensitised humans. An LOAEL of 4.3 µg/kg body weight per day was established as a reference point (EFSA, 2020a). The MOE approach of at least 30 applies.
EFSA has established a TDI of 13 µg/kg body weight per day for chronic intake. The critical effect for this was the effect on reproduction (increased post-implantation loss) in rats with a $BMDL_{10}$ of 1.3 mg/kg body weight per day (EFSA, 2020a).

[30] $BMDL_{01}$ is the 95% lowest confidence interval of the estimated dose which results in 1% added risk.
[31] $BMDL_{10}$ is the 95% lowest confidence interval of the estimated dose which results in 10% added risk.
[32] $BMDL_{0,5}$ is the 95% lower confidence interval of the estimated dose which results in 0.5% added risk.

Exposure estimate

WFSR found levels of cadmium, lead, mercury, copper, a total of organic and inorganic arsenic and nickel in the liver and kidneys of the animals of ten areas along Waal (Beuningen and Loevestein), the Rhine (Millingen, Ooijpolder, Meinerswijk, Blauwe Kamer, Elst, Prins Willem III plantation (PWIII) and Amerongen) and along the Meuse (Koornwaard) (Hoogenboom et al., 2022b). Given that heavy metals tend to be more concentrated in organs than in meat, these tissues were first measured and compared to the ML, if available. Operators no longer offer offal for consumption purposes, which is why the levels in meat were also tested for the animals in which the highest levels were found in liver and kidney. The levels in kidney and liver were determined from a total of 42 animals. The levels in meat were also measured for twenty animals.

BuRO summarises the findings pertaining to the measurements of heavy metals as follows.

- The ML for cadmium was exceeded in nine kidney samples (1.3-8.2 mg/kg); all samples were from animals older than two years. Cadmium increases in bovine kidneys in proportion to exposure time(BuRO, 2008), which is why the sale of kidneys of bovine animals older than two years is not permitted in the Netherlands. The cadmium level in the liver of one animal exceeded the ML, at 0.7 mg/kg. The level in the meat of all animals was below the quantification limit of the analytical method (LOQ) of 0.006 mg/kg.
- At the time of the study, lead levels exceeding ML were found in the liver and kidney of one animal. This conclusion was made on the basis of the ML in force in 2021, i.e. 0.5 mg/kg. Based on the new, lower ML, i.e. 0.2 mg/kg, six liver samples (0.23-0.51 mg/kg) and seven kidney samples (0.25-0.62 mg/kg) exceeded the limit. In all meat samples that had been tested, the lead levels were below the LOQ of 0.008 mg/kg.
- The ML for mercury was exceeded in some minor cases in the liver (one animal; 0.027 mg/kg) and kidney (two animals; 0.27 and 0.29 mg/kg). The concentrations in meat of all sampled animals did not exceed the ML. There was one animal in which an extremely low level of 0.006 mg/kg was found in meat, well below the ML. In all other meat samples, the levels were below the LOQ of 0.003 mg/kg.
- The level of copper in the liver of two animals exceeded the ML at 34 and 42 mg/kg. The level of copper in the kidneys (2.4-4.6 mg/kg) and meat (0.42-1.9 mg/kg) did not exceed the respective MLs.
- Arsenic was found in detectable amounts in fifteen samples, >0.007 mg/kg. The range of positive measurements is between 0.0071 and 0.11 mg/kg with an average of 0.04 mg/kg. As there are no statutory maximum levels in bovine meat, the levels found cannot be compared with it.
- In all meat samples, nickel levels were below the LOQ of 0.02 mg/kg. In six kidney samples (0.02-0.05 mg/kg) and one liver sample (0.025 mg/kg), the level was just above the LOQ. No ML has been established for nickel to which these levels can be compared.

MLs exceeded in liver and kidney were measured in animals from different areas; there is no clear correlation between origin and measured levels. All meat samples meet the MLs for cadmium, lead, mercury and copper. Nickel is not detectably present in meat. Arsenic was found in a number of meat samples, the highest level measured at 0.11 mg/kg. Table 10 shows the highest measured levels of the different metals in meat.

Table 10: An overview of the highest levels of metal measured in wilderness meat.

	Highest level measured in meat (mg/kg)	Statutory limit (mg/kg)	Origin of the animal with the highest measured level
Cadmium	<0.006	0.05	All levels <LOQ
Lead	<0.008	0.1	All levels <LOQ
Mercury	0.006	0.01	Millingen
Copper	1.9	5	Koornwaard
Arsenic (total)	0.11	n/a	Loevestein
Nickel	<0.02	n/a	All levels <LOQ

Indication of levels of arsenic in meat

Given that the levels of arsenic cannot be compared to an ML, BuRO has collected additional information about arsenic in foodstuffs. First, the levels of arsenic found in wilderness meat were compared with the levels in other game meat, as these animals also spend long periods of time in the outdoors and therefore come into contact with environmental contaminants. BuRO does not have any data on levels of arsenic in regular bovine meat.

A comparison with available data from the KAP[33] database from 2017 to 2019 shows that the average level of 0.040 mg arsenic/kg in wilderness meat is in the range of levels in meat from other wild animals such as deer, boar and roe deer. The maximum level of 0.11 mg/kg is close to the maximum level found in wild boar. As such, the arsenic levels in wilderness meat are no higher than in meat from wild animals grazing and rooting outdoors for long periods of time. See table 11.

Table 11: An overview of the measurement data on arsenic in the KAP database between 2017 and 2019 and the measurements made by WFSR in wilderness meat (Hoogenboom et al., 2022b).

	Number exceeding LOQ[1] (total)	Lowest level measured (mg/kg)	Highest level measured (mg/kg)
Imported game	0 (8)	<LOQ	<LOQ
Wild venison	2 (44)	0.010	0.027
Wild roe venison	4 (49)	0.008	0.058
Meat of wild boar	9 (39)	0.010	0.140
Farmed venison	1 (6)	0.011	0.011
Wilderness meat (current study)	15 (20)	0.007	0.110

[1] LOQ is the quantification limit of the analytical method

A study carried out by RIVM on Dutch intake of contaminants through the consumption of food according to the food-based dietary guidelines for the Netherlands (‘Schijf van Vijf’, Dutch Wheel of Five guidelines), which is thus a fictitious consumption pattern, shows that the main intake sources of inorganic arsenic are fish, rice and drinking water (Boon et al., 2017). In 2014, EFSA calculated the European population’s chronic intake of the total and the inorganic arsenic (EFSA, 2014). For this purpose, EFSA used both food consumption data from the EFSA Comprehensive Database and national food consumption data. For all age groups except infants and toddlers, cereals and cereal products were the dominant contributors. These products were non-rice-based, mainly wheat bread and rolls. Other food groups contributing to inorganic arsenic intake were rice,

[33] The Quality Programme for Agricultural Products (KAP) database has data on the presence of residues and contaminants in food and feed, measured by WFSR on behalf of the government.

milk and milk products and drinking water. Meat does not appear to be a major source of arsenic intake.

Levels of heavy metals in soil and grass

WFSR also measured the levels of heavy metals in soil and grass in the floodplains at Beuningen. The levels were higher in soil than in grass. The levels in grass were also compared with a sample from outside the floodplain (monitoring area). There were no distinct differences between the levels in grass in the floodplains and in the monitoring area. There were two areas within the floodplain that showed higher levels of arsenic in the grass, but nowhere else in other areas. Levels of heavy metals in grass were strongly elevated just after a flood, presumably due to deposition of contaminated silt. Due to the lack of measurement in the soil of the monitoring area, no comparison can be made between levels of heavy metals in the soil of the floodplain and the monitoring area.

Risk characterisation

Offal

Since operators no longer offer organ meat from these animals for consumption purposes, the MLs exceeded in liver and kidney samples will not lead to health risks for consumers of wilderness meat.

Meat

All meat samples amply complied with the applicable statutory maximum levels for cadmium, lead, mercury and copper. It can thus be concluded that wilderness meat meets the requirements imposed on bovine meat for metals. Nickel was not detectably present in meat and will thus not lead to any consumer health risks.

The levels of arsenic cannot be assessed against a statutory maximum level, which is why BuRO carries out a risk assessment to indicate the levels in wilderness meat. A study carried out by RIVM on the Dutch intake of contaminants through the total diet according to the food-based dietary guidelines for the Netherlands ('Schijf van Vijf', Dutch Wheel of Five guidelines), which is thus a fictitious consumption pattern, shows that the main intake of arsenic from the total diet approximates the $BMDL_{0.5}$of 3 μg/kg body weight per day (derived from JECFA) (Boon et al., 2017). The margin of exposure (MoE) is therefore 1. This study concluded that there may be potential health risks for the Dutch situation (Boon et al., 2017). Also, calculations with Dutch food consumption data by EFSA show that the MoE between the BMDL of$_{0.5}$ and the P95 intake from the total diet is small, namely three to seven depending on the age group (EFSA, 2014).

Under the assumption that arsenic exposure comes solely from wilderness meat, for a 60-kg adult, the BMDL of$_{0.5}$, taking into account a margin of exposure of 10, is exceeded at a daily intake of 18 μg (0.3 μg/kg body weight per day x 60 kg). Consumption of meat with the highest measured level of 0.11 mg/kg leads to the following intake: A consumption of 110 g of wilderness meat with a level of arsenic of 0.11 mg/kg per week results in an intake arsenic of 1.7 μg/day (0.03 μg/kg body weight per day) for a 60-kg adult. A consumption of 300 g results in an intake of arsenic of 4.7 μg/day (0.08 μg/kg body weight per day). Consumption of wilderness meat thus contributes relatively little to the total tolerable intake of arsenic. All of the arsenic present here is taken to be in inorganic form, which is the most toxic form. Intake of arsenic from wilderness meat therefore does not lead to health risks for consumers. It should be noted, however, that this exposure comes on top of the intake from other foods, which stands at a high intake for the Dutch situation. It is unclear whether or not the consumption of wilderness meat leads to added health risks.

Based on the above information, BuRO concludes that the presence of heavy metals in wilderness meat complies with the MLs imposed on bovine meat. The intake of arsenic from wilderness meat does not lead to health risks. However, due to the absence of data on levels of arsenic in regular beef, it is not clear whether the consumption of wilderness meat leads to a higher exposure to arsenic when compared to regular beef.

Conclusion
The level of heavy metals in organs exceeds the ML in a number of samples. However, as operators no longer offer organ meat from these animals for consumption purposes, there are no health risks for wilderness meat consumers. The levels in meat are below the ML or below the LOQ, except for arsenic. Wilderness meat therefore meets the MLs imposed on bovine meat for cadmium, lead, copper and mercury. Nickel is not detectably present. The exposure to arsenic from wilderness meat does not in itself lead to health risks. However, it is unclear whether or not the consumption of wilderness meat leads to a higher intake than the consumption of regular beef.

PART 3: POLY- AND PERFLUOROALKYL SUBSTANCES (PFASs)

Legal framework

MLs for foodstuffs have currently been established for poly- and perfluoroalkyl substances (PFASs). These MLs are expected to come into force in 2023. MLs will apply to perfluorooctane sulphonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorohexane sulphonic acid (PFHxS) and the sum of these four substances in bovine meat and organs, among others. These values are based on levels that have been reported to date.

There are restrictions for the application of a number of PFOS and PFOAs. Other PFASs are on the Community Rolling Action Plan (CoRAP) list for substance assessments of the European Chemical Agency, ECHA, or on the REACH Candidate List for Substances of Very High Concern. A broad restriction of PFASs under REACH is currently under preparation with the involvement of the Netherlands, among others (ECHA, 2022).

Hazard identification

PFAS is a group name for fluorinated aliphatic hydrocarbons. PFASs are man-made substances that are not naturally present in the environment. There are over 4,000 known PFASs (OECD, 2018).

PFASs are chemically and thermally stable substances, which is why they are used as coatings in many industrial and consumer products. Examples include furniture fabric, outdoor and rainwear, and food packaging materials (food contact materials). Because of their stability, PFASs also remain present in the environment and the food chain for long periods of time and some PFASs accumulate in humans and animals. The use of PFASs in many products and industrial emissions and incidents have led to their release into the environment in soil, sludge and surface water, among others. PFAS compounds are relatively soluble in water, allowing these compounds to spread easily into the environment via water and aerosols.

Upon the European Commission’s request, EFSA carried out a scientific evaluation of human health risks of 27 different PFASs present in food (EFSA, 2020b). The PFASs evaluated included perfluoroalkyl carboxylates (PFCAs) and perfluoroalkyl sulphonates (PFSAs).

Hazard characterisation

Most of the 27 PFASs from EFSA’s recommendations are easily absorbed via the gastrointestinal systems in mammals, including human beings (EFSA, 2020b). PFASs then spread to plasma and other parts of the body. Depending on the type of PFAS, this may also mean accumulation in the liver and blood. PFASs are excreted via urine but probably also via faeces, although this has hardly been examined yet. PFCAs and PFSAs are not metabolised by humans or animals. Conversely, precursors such as fluorotelomer alcohols (FTOHs) and polyfluorinated alkyl phosphate esters (PAPs) can be transformed into metabolites through biotransformation including PFCAs, while other precursors are converted into PFSAs. Human half-lives for PFASs depend on the type of PFAS. The estimated half-lives of short-chain PFASs, such as PFBA, PFBS and PFHxA, range from a few days to a month. The half-lives of long-chain PFASs, such as PFOA, PFNA, PFDA, PFHxS or PFOS, amount to several years.

PFASs are not acutely toxic, which is why EFSA has not derived an acute reference dose (ARfD). EFSA did derive a TWI for the sum of four PFASs for chronic effects: PFOA, PFOS, PFNA and PFHxS (EFSA-4) (EFSA, 2020b). At present, these are the four PFASs that contribute most to the levels measured in human serum.

In humans, these four PFASs have similar toxicokinetic properties, similar accumulation and long-term half-lives. EFSA concluded that the effect on the immune system is the critical effect. A German study, in which diphtheria antibody formation was contrasted with serum levels of these four PFASs, derived a $BMDL_{10}$ (benchmark dose lower confidence limit)[34] of 17.5 ng/ml for a one-year-old child. EFSA then used a PBK model to estimate[35] the required intake of the four PFASs by mothers breastfeeding their child during the first twelve months. This modelling was used to derive how much PFASs a mother has to ingest to reach a serum concentration of 17.5 ng/ml in a one-year-old child. It proved to be a daily intake of 0.63 ng/kg body weight per week. This value resulted in a TWI of 4.4 ng/body weight per week. EFSA did not factor in any additional uncertainty assessors, as the $BMDL_{10}$ is based on children and as reduced vaccination response is considered a risk factor for diseases rather than a disease itself. This TWI also protects against other described health effects, such as increased cholesterol and ALT concentration in serum and reduced birth weight.

<u>Application of the EFSA TWI in a risk assessment</u>

As the TWI is based on the sum of PFASs, it raises questions regarding the application of this health-based guidance value in a risk assessment. These four PFASs are not the only PFASs present in food, drinking water and soil, among others. It may also be the case that the concentration of only one of the four PFASs is known in food, for example. There are two possibilities of incorporating this TWI in the risk assessment:

1. Concentration addition

In applying the TWI, EFSA assumes equipotency; equal potency of the EFSA-4 with respect to the toxicological effect on the immune system. EFSA's analysis showed that there was insufficient data to determine the relative potency factors[36] (RPFs) for the individual PFASs in relation to the critical effect (EFSA, 2020b). Following this assumption, BuRO can only apply the EFSA TWI in a risk assessment whereby the concentration of one or more of the four PFASs is known. This approach has two limitations for the risk assessment:

- Other PFASs cannot be tested against the TWI. EFSA does indicate that some of these substances are likely to cause similar effects but due to the absence in the children's blood in the critical study, they could not be included in the TWI. These other PFASs need to be assessed according to health-based guidance values specifically derived for these individual substances. These are not available for all known PFASs. Furthermore, deriving a health-based guidance value may lead to an underestimation of the risk based on effects that occur at much higher doses.
- Equipotency assumed that these four PFASs share the same toxicity. However, there are likely to be differences in the four PFASs' potency. EFSA indicates that there are insufficient data at present to correct this.

2. RPF method

With respect to the question as to how the EFSA TWI should be applied in a risk assessment, RIVM drew up a memorandum (RIVM, 2021). RIVM suggests the use of RPFs, because:

[34] $BMDL_{10}$ is the 95% lowest confidence interval of the estimated dose which results in 10% added risk.
[35] A PBK model is a kinetics model based on human physiology. Computer modelling is used to model the toxicokinetic of a substance and estimate the intake leading to a particular serum level in humans.
[36] Relative Potency Factors indicate the degree of hazardousness of substance A, B or C relative to an index substance.

- the method can be applied to individual PFASs, EFSA's four PFASs and other PFASs which EFSA has not included in the TWI.
- the method can assess PFAS mixtures in different ratios.
- the method includes any differences in the potency between PFASs.
- the method is conceptually simple and practically applicable.

RPFs present the toxic potency of the individual PFAS against PFOA (index substance). RIVM has currently derived an RPF for 23 PFASs based on liver effects (Bil et al., 2021; RIVM, 2021). This is a different effect from the immune effects on which the EFSA TWI is based. RIVM argues that in the absence of immune-specific factors from studies with humans, RPFs can also be derived based on other effects. The application of the current RPF values in a broader context requires a validation of this calculation method (Bil et al., 2021; RIVM, 2021). The RIVM's RPFs can be used to convert an individual PFAS concentration in, for example, meat into PFOA equivalents (PEQ), which can then be compared with the EFSA TWI. For example, an analysis result of bovine meat is made up of a combination of three PFASs (A, B and C). PFAS A is PFOA and has an RPF of 1, which is multiplied by the amount of A that is present. PFAS B and PFAS C have an PRF of 2 (B) (more potent than PFOA) and 0.01 (C) (less potent than PFOA), which are multiplied by the amount of B and C that is present. The levels of A, B and C are then added and expressed in 'x unit' PEQ in order to make it possible to evaluate the toxicity of the mixture as though it only contains PFOA.

In light of the fact that there is at present no consensus yet on the approach to calculating PFAS concentrations, BuRO will calculate the exposure in this risk assessment based on both concentration addition and the RPF method.

Exposure estimate
WFSR has examined the levels of twenty PFASs in cattle from a total of twelve areas along the Waal (Beuningen and Loevestein), the Rhine (Millingen, Ooijpolder, Meinerswijk, Blauwe Kamer, Elst, Prins Willem III plantation (PWIII) and Amerongen), the Meuse (Border Meuse and Koornwaard) and Merwede (Noordwaard) (Hoogenboom et al., 2022b). First, the levels in liver and meat were determined. Kidney concentrations were also measured for some of the animals with the highest liver levels. Compared to the liver, which is known to have the highest levels of PFASs, the levels of PFOS in kidneys were found to be on average a factor of 4 lower; for meat, the difference was on average a factor of 47.

The levels of PFOS were highest in the liver of the cattle that had been tested. This is in line with the fact that PFOS heavily accumulate in bovine animals (Hoogenboom et al., 2022b). PFOA was found in part of the livers tested, particularly in the livers of bulls. PFNA and PFDA were also detectably present in the liver, and also PFUnDA and PFDoDA had been measured in some animals. WFSR did not find PFHxS, short chains of PFCAs and PFSAs and GenX. The highest levels were found in wild cattle from the floodplains along the Waal and the Rhine. The WFSR report provides a full overview of the measurements (Hoogenboom et al., 2022b).

This assessment compares the levels of PFOS, PFOA, PFNA and PFHxS in wilderness meat based on concentration addition and RIVM's RPF method with the EFSA TWI. The levels in liver and kidney are not relevant for consumers' health as operators no longer offer these organs for consumption purposes. Also, there were no MLs for levels of PFASs in meat in place yet at the time of the study. Still, a first comparison of the levels found, thereby taking into account a measurement uncertainty of 40%, shows that about half of the liver samples tested exceed the recently established MLs for PFOS and/or PFNA and for the sum of the four PFASs.

(Hoogenboom et al., 2022b). In addition, one kidney sample exceeds the established ML for PFOS.

Levels of PFOS, PFOA, PFNA and PFHxS in wilderness meat

Only PFOS was found in meat (Hoogenboom et al., 2022b). The levels of PFOA, PFNA and PFHxS were below the detection limits of 0.10, 0.15 and 0.05 ng/g respectively. BuRO calculated the sum of the four PFASs according to two scenarios, i.e. a lower-bound (LB) scenario[37] and an upper-bound (UB) scenario[38]. See Table 12. BuRO determined the sum of the four PFASs based on concentration addition (equipotency) and according to the RPF method. RIVM derived the following RPFs for these PFASs: PFOA: 1 (index substance); PFOS: 2; PFNA: 10 and PFHxS: 0.6 (RIVM, 2021). The weighted sum is then expressed as equivalents of the PFOA reference substance (PFOA equivalents; PEQs). Given that only PFOS was detectably present, only the PFOS concentration determines the concentration of the EFSA-4 in the LB scenario. RIVM also derived RPFs for other PFASs but these PFASs had not been detected in meat. The RPF method can principally include 23 PFASs. BuRO also limits itself with the RPF method to the EFSA-4, as the risk in the UB scenario would otherwise be too dependent on the high LOQs of the non-detected PFASs in wilderness meat.

[37] The sum of the four PFASs whereby levels of individual PFASs below LOQ are set at zero.
[38] The sum of the four PFASs whereby the levels of individual PFAS below LOQ are made equal to the LOQ.

Table 12: Range of levels of PFASs (EFSA-4) measured by WFSR in wilderness meat at the locations examined. Given that only PFOS was detectably present, the PFOS concentration equals the concentration of the EFSA-4 in the LB scenario. In all samples, the levels of PFOA, PFNA and PFHxS were below the LOQ of 0.10, 0.15 and 0.05 ng/g respectively. The LOQ of PFOS is 0.10 ng/g.

Area	**Number of animals**	**EFSA-4 level (LB) (ng/g)**	**EFSA-4 level (UB) (ng/g)**	**EFSA-4 level (LB) level (ng PEQ/g)**	**EFSA-4 level (UB) level (ng PEQ/g)**
		Concentration addition		**RPF method**	
De Waal					
Beuningen	3 cows	0.43-0.70	0.73-1.0	0.86-1.40	2.40-2.94
Loevestein	5 bulls 1 cow	0.17-0.92	0.47-1.22	0.34-1.84	1.88-3.38
Gendtse Waard	1 bull 1 cow	0.11-0.22	0.41-0.52	0.22-0.44	1.76-1.98
The Rhine					
Millingen	3 cows	0	0.40	0	1.74
Ooijpolder	6 cows	0-0.29	0.40-0.59	0-0.58	1.74-2.12
Meinerswijk	3 bulls	0.19-0.27	0.49-0.57	0.38-0.54	1.92-2.08
Blauwe Kamer	6 bulls	0.16-0.63	0.46-0.93	0.32-1.26	1.86-2.80
Elst (Utrecht)	3 cows	0.63-0.94	0.93-1.24	1.26-1.88	2.80-3.42
PWIII	3 cows	0.20-0.94	0.5-1.24	0.4-1.88	1.94-3.42
Amerongen	3 cows	0.10-0.19	0.40-0.49	0.20-0.38	1.74-1.92
The Meuse					
Border Meuse	2 bulls	0	0.4	0	1.74
Koornwaard	7 cows 2 bulls	0-0.10	0.4-0.5	0-0.20	1.74
De Merwede					
Noordwaard	2 cows	0.42-0.48	0.72-0.78	0.84-0.96	2.38-2.50
Total	51				

[1] The detection limit for PFOS is 0.10 ng/g

An initial comparison of the levels of PFAS measured in wilderness meat with the recently established MLs shows that for ten out of a total of 51 animals, from floodplains along the Waal and Rhine, the ML for PFOS is exceeded (Hoogenboom et al., 2022b). This comparison also took a measurement uncertainty of 40% into account.

Levels of PFAS in soil, grass and water

WFSR determined levels of PFASs in the soil, grass and water in the floodplains at Beuningen and Loevestein (Hoogenboom et al., 2022b). One in seven grass samples showed a detectable presence of PFOS. PFOS was measured in nearly all soil samples in the floodplains. These levels were not much higher than the control sample taken outside the floodplain of Beuningen. One in ten soil samples also measured PFOA. According to WFSR, the levels of PFOS and PFOA in the river water of the Waal near Beuningen and two small lakes in the floodplains of Beuningen proved comparable to levels in surface water that had previously been reported. These levels also more than met the environmental quality standards and maximum acceptable concentrations for freshwater.

This limited data set from WFSR therefore offers not clear explanation for the levels of PFAS in meat.

Seasonal factors on levels of PFAS and decreases following relocation to a clean environment

WFSR determined levels of PSAS in meat in cattle from the Loevestein area after the winter period (three animals in April 2021) and after the summer (two animals in November 2021) (Hoogenboom et al., 2022a; Hoogenboom et al., 2022b). Although it is known that PFOS accumulates heavily in bovine animals, the levels measured in wilderness meat after the summer period, i.e. 0,24 and 0,17 ng/g, were a factor of 4 lower than the levels measured after the winter period, at 0.74, 0.91 and 0.92 ng/g. As a possible explanation, WFSR suggests this to be related to a lower exposure during the summer and fall period. The levels in the livers of these animals were also substantially lower, both for PFOS as well as for PFNA.

The course of the levels of PFAS in blood was determined for the five Rode Geuzen who had been rehomed from the Loevestein area to a stable. For further details see part 1 of this substantiation (Hoogenboom et al., 2022a). At the start of the experiment, only PFOS could be measured in these animals' blood (March 2021). Two months after having been stabled, the levels of PFOS dropped by 46%. After seven months, the reduction was 67%. After the slaughter, the meat of two animals showed a detectable presence of PFOS, at 0.11 and 0.15 ng/g respectively. For the other three animals, the level in meat was below the detection limit of 0.10 ng/g. WFSR attributes the decrease largely to elimination of exposure in combination with growth dilution (factor 2 increase in weight) and to a lesser extent to excretion.

The relocation of four oxen of different breeds of cattle from De Bakenhof floodplain near Arnhem to a clean farm in Elst, Gelderland, showed a drop in levels of PFOS in blood of approximately 47% in four months' time (Hoogenboom et al., 2022a). Similar to the dioxins measured in these animals (see part 1 of this substantiation), after this period, after which the animals were moved to another pasture, the blood levels actually showed a slight increase again. It is unclear whether higher levels of PFOS were present in this second pasture.

Intake calculations for PFASs in wilderness meat

Three factors depend on the intake calculation for PFASs by the consumption of wilderness meat: the levels of PFASs in meat, the amount of meat eaten and the frequency with which it is eaten. The intake calculation starts from P50 levels of the sum of PFOS, PFOA, PFNA and PFHxS. See Table 13. It is not likely that consumers will eat wilderness meat with P95 levels for long periods of time. BuRO therefore considers the P95 levels too conservative for the intake calculations.

BuRO calculated the levels based on WFSR's 51 measurement results in wilderness meat. See Table 12. This approach provides a representative overview of the levels in wilderness meat eaten by consumers on a long-term basis, as this meat comes from different areas in the Netherlands (Front office, 2021). To calculate weekly consumption amounts, BuRO takes an average of 110 grams per week and 300 grams per week for large eaters. An explanation of these consumption amounts is elaborated in part 1 of this substantiation.

Determination of levels of PFAS in wilderness meat

Based on a Jarque-Bera test of normality, with a P-value of 0.05, BuRO concludes that the data on concentration (51 data points) from the WFSR study are not normally distributed. The P50 and P95 are therefore determined after a square root transformation of the data set; logarithmic transformation, as was done in part 1 for the dioxins and dioxin-like PCBs, is not possible on account of the zero values in the data set in the LB scenario.

Table 13: The P50 and P95 levels calculated by BuRO for the sum of PFOS, PFOA, PFNA and PFHxS (EFSA 4) in wilderness meat.

Methodology	**P50 level**		**P95 level**	
	LB	**UB**	**LB**	**UB**
Concentration addition (ng/g)	0.2	0.5	0.9	1.2
RPF method (ng PEQ/g)	0.4	1.8	1.9	3.4

<u>Comparison of the levels of PFAS in wilderness meat and regular beef</u>

Since 2017, WFSR has a monitoring project in which levels of PFAS in animal products are being examined (Hoogenboom et al., 2022b). So far, 43 bovine samples have been tested; all levels were below the LOQ. During the project, the detection limits for PFOS were reduced from 5 ng/g to 0.1 ng/n and for some of the measurements, the detection limit was thus higher than the levels currently found in wilderness meat. Based on twelve measurements in the livers of regular cattle, which found levels between 0.3 and 1.4 ng/g, the PFOS level in this cattle from the floodplains appears to be higher, at 1.75 and 65.75 ng/g in liver.

<u>Weekly intakes of PFASs from wilderness meat by adults</u>

Based on the FO assessment (Front office, 2021) on the consumption of wilderness meat, BuRO assumes that a wilderness meat consumer eats between 110 and 300 grams of wilderness meat per week, 110 grams being the average target group consumption.

Based on the data above, BuRO establishes two intake scenarios for adults:

- Scenario 1: consumers of wilderness meat eat 300 grams of meat per week with a median level of PFASs.
- Scenario 2: consumers of wilderness meat eat 110 grams of meat per week (average quantity) with a median level of PFASs.

These intake scenarios do not take any exposure from other sources into account. The total weekly intake of PFAS is calculated according to the following formula:

$$\frac{(consumption\ amount\ x\ level\ of\ PFAS)}{body\ weight}$$

Depending on the scenario, the individual components of the formula are filled in as follows:
- Consumption amount: 110 g/week or 300 g/week
- Level of PFAS: LB P50 levels for the risk assessment
- Body weight: 60 kg

The results of the intake calculations are provided in Table 14. Applying the RPFs, the intake in the LB scenario is about two times higher compared to the concentration addition approach.

Table 14: Overview of the weekly intake of PFASs from wilderness meat for an adult (per kg body weight) based on the P50 PFASs levels. The intake is expressed in ng/week per kg of body weight

Meat consumption (g/week)	**Concentration addition**		**RPF method**	
	LB	**UB**	**LB**	**UB**
300	1	2.5	2	9.5
110	0.4	0.9	0.7	3.5

Intake calculations with the UB scenario include the uncertainty that arises due to the fact that the different PFASs may be present in levels below the detection limit, thus providing an overestimation of the weekly intake. BuRO therefore uses the calculations of the UB scenario in the uncertainty analysis of the risk assessment. The UB scenario, and hence the corresponding intake, are a factor of 1.3 to 5 higher compared to the LB scenarios.

Weekly intakes of PFASs from wilderness meat by children
FO concludes that children are also likely to eat wilderness meat (Front office, 2021). The consumption amounts are unknown however, which is why BuRO calculates the amount of wilderness meat which a child of 12 kg can eat on a weekly basis before exceeding the TWI. BuRO then checks whether this scenario is realistic and whether the presence of PFASs in wilderness meat can lead to health risks. This does not take account of exposure from other sources. Children with a body weight of 12 kg may ingest 53 pg PFAS per week, which is 4.4 ng/kg body weight/week x 12 kg, before the TWI is exceeded. The weekly amount of meat causing to exceed the TWI is calculated according to the following formula:

$$\left(\frac{53\,ng}{Level\ of\ PFAS}\right)$$

The levels of PSAS used for the calculations are shown in Table 13. Table 15 gives an overview of the calculated weekly consumption amounts whereby the TWI is exceeded.

Table 15: Overview of the weekly consumption amounts of wilderness meat for a child of 12 kg whereby the TWI is exceeded at different P50 PFAS levels.

Methodology	Wilderness meat consumption (g/week)	
	LB	UB
Concentration addition	264	106
RPF method	132	28

Risk characterisation
In a risk characterisation, the exposure estimate is compared to the health-based guidance value. In this case, the health-based guidance value is the TWI of 4.4 ng/kg body weight/week as established by EFSA (EFSA, 2020b). For a 60-kg adult, the tolerable upper intake level for PFASs comes down to 264 ng per week, this being 4.4 ng/kg body weight/week x 60 kg).

Only PFOS was detectably present in wilderness meat. This means that the levels of the sum in the LB scenario are solely determined by PFOS, as the LB scenario sets non-detectable levels equal to zero. The levels in the UB scenario are also determined by the detection limits of the analytical method of non-detectable PFASs, the actual level is between zero and the detection limit. The levels in the LB scenario present a more realistic estimate of the intake calculation for the risk assessment in this case as the levels for three out of the four substances in all samples lie below the detection limit. The use of the LB scenario in the risk assessment could lead to a possible underestimation; this uncertainty is discussed below.

Table 16 presents the ratio of the TWI and the calculated intake for an adult person for the different intake scenarios. A ratio of more than 1 exceeds the health-based guidance value, which means that a consumer health risk cannot be ruled out. The exposure from other sources has not been included in this approach.

Table 16: The TWI's ratio and the calculated weekly intake of PFASs from wilderness meat with a P50 level for 60-kg adults.

Meat consumption (g/week)	Concentration addition		RPF method	
	LB	UB	LB	UB
300	0.2	0.6	0.5	2.2
110	0.1	0.2	0.2	0.8

In the LB scenario, the TWI is not exceeded when large amounts of 300 grams of wilderness meat is consumed on a weekly basis. The highest contribution of wilderness meat to the tolerable upper intake levels for PFASs is 50% in case of 300 grams of wilderness meat per week, thereby taking into account the RPFs for the concentration calculation. The PFAS levels in wilderness meat alone therefore do not lead to health risks for adult consumers, however it does substantially fill up the tolerable upper intake level.

In the UB scenario, the TWI is exceeded only for large consumption amounts of 300 grams per week and when RPFs are applied for the concentration calculation. The UB scenario includes the uncertainty in the risk assessment due to the possible presence of PFASs below the LOQ. These calculations can therefore conclude that lower detection limits are needed to definitively assess the risks of high consumption levels.

When children of 12 kg consume 132 or 264 grams of wilderness meat on a weekly basis (depending on the method used for determining the PFAS concentration), the TWI is exceeded in the LB scenario. See Table 15. These numbers are based on P50 PFAS levels. According to RIVM's Dutch National Food Consumption Survey, the average daily beef consumption of one-to-three-year-olds is 3.3 grams a day and the P95 consumption is 21 grams a day[39]. It is thus likely that a child could eat 147 grams of wilderness meat per week (P95 consumption amount of beef per week). This means that the consumption of wilderness meat with LB P50 levels could potentially lead to health risks for children up to 12 kg, if RIVMS' RPF method for the concentration calculation is used. Taking concentration addition, the tolerable upper intake level for children will be filled up for the most part.

For the UB scenario, the safe consumption amount of wilderness meat with P50 PFAS levels is also less than 140 grams, even if concentration addition is used in the concentration calculations. Given that these are realistic consumption amounts, the conclusion can be made based on the UB scenario that lower detection limits are needed to definitively assess the risks for children.

<u>The intake of PFASs from the total Dutch diet</u>
In an advice on a Dutch drinking water reference value for PFASs, RIVM concluded in 2021 that the intake of PFOS, PFOA, PFNA and PFHxS from the total Dutch diet and drinking water exceeds the TWI of 4.4 ng/kg body weight per week (Van der Aa, 2021). By exceeding the TWI, the intake of PFASs from the total Dutch diet and drinking water exceeds the TWI and could therefore have potentially detrimental effects on health. RIVM's calculations are considered to be indicative as these are based on PFAS measurements from 2009. New measurements are needed for a better estimate. Also, the intake calculations made by EFSA in 2020 show that the intake of PFASs from the total diet is too high for part of the population (EFSA, 2020b).

[39] https://statline.rivm.nl/

Based on a limited data set, the provisional conclusion can be drawn that wilderness meat may potentially contain more PFASs than regular beef (Hoogenboom et al., 2022b). The consumption of wilderness meat would in that case lead to a higher PFAS intake compared to regular beef.

Conclusion

PFOS is found in wilderness meat and these levels are significantly lower than the levels measured in liver and kidneys. Approximately half of the livers measured had a higher level than the ML recently established for PFOS and/or PFNA and higher than the set ML for the sum of four PFASs. In wilderness meat, the ML established for PFOS is exceeded in ten of the 51 animals tested. Other PFASs were also found in liver and kidneys. Little is known about levels of PFAS in farm cattle. Based on twelve measurements in the livers of farm cattle, the PFOS level in the wild cattle from the floodplains appears to be higher.

A limited number of measurements in soil, grass and water show no clear explanation for the elevated levels of PFAS in wilderness meat. Based on the levels measured in cattle from the Loevestein floodplains there appear to be seasonal factors affecting PFAS levels in liver and meat. The levels in meat were a factor 4 lower after the summer period in comparison with meat of animals that had been slaughtered after the winter period.

Based on P50 levels and the lower-bound scenario, the risk assessment shows that wilderness meat's highest contribution to the tolerable upper intake levels for PFAS is 50%. This is based on the consumption of 300 grams of wilderness meat per week and the RPFs have been applied for the concentration calculation. The PFAS levels in wilderness meat therefore do not lead to health risks for adult consumers. The consumption of reasonable quantities of wilderness meat exceeds the tolerable upper intake levels for children (12 kg), if RIVM's RPF method for concentration calculations is used. This means that the consumption of wilderness meat with LB P50 levels could potentially lead to health risks for children. Taking concentration addition, the tolerable upper intake level for children will be filled up for the most part.

Calculations with the UB scenario show that lower detection limits are needed to definitively assess the risks of high consumption levels for adults. The same applies to the intake scenario of children on the basis of concentration addition.

The intake of PFASs from other sources have not been included in this risk assessment. The indicative calculations from RIVM and the intake calculations from EFSA show that the intake of PFASs from the total Dutch diet and drinking water is too high. This is based on regular beef consumption; the intake from wilderness meat may be higher.

PFOS levels in the blood of animals that have been rehomed to a clean environment can halve in a few months, mainly by reducing exposure combined with growth dilution, and to a lesser extent by excretion.
Based on a limited number of measurements, the levels in meat of animals that had been slaughtered after the summer period were also a factor 4 lower in comparison with meat of animals that had been slaughtered shortly after the winter period.

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Appendix 1: search strategy of the literature review

BuRO has searched for scientific and grey literature on the toxicology of dioxins, levels of dioxins in foodstuffs and soil levels in relation to consumers' health risks. Given that EFSA included relevant general, toxicological information and data on levels in foodstuffs in their memorandum at the end of 2018, BuRO used this document as the basis. Additionally, BuRO specifically searched the RIVM website on any relevant documents covering this subject. BuRO also requested an overview from WFSR on the monitoring programme of dioxins in Dutch bovine meat.

PubMed and Google were used in the search for literature on levels of dioxins in soil in relation to consumers' health risks. Use was made of different combinations of search terms: dioxins, floodplain or floodplains, soil levels / soil content, grass levels / grass content, meat, milk, cattle, beef. The search terms in Google were both in English and Dutch. There appeared to be a limited number of studies available that have been summarised and highlighted in a 2013 RIKILT report and this report was used as a basis by BuRO.
An EFSA/WFSR expert was then consulted to check whether BuRO had found the most relevant literature on dioxins.

For information about the heavy metals and PFAS, BuRO made use of the most recent EFSA recommendations on the respective substances. BuRO specifically searched the EFSA website for this. This information is supplemented by relevant sources that had already been processed in BuRO's recent work. Additional information on the monitoring programme into PFAS was obtained from WFSR.

BuRO consulted the websites of operators for general information on wilderness meat.

Appendix 2: calculation on fat percentages of meat products

Mixed beef package

	grams of meat	% of fat, unprepared (Dutch Food Composition Table (NEVO)		grams of meat	% of fat (average)
Minced meat	1,500	16.5		247.5	
sausage	560	14.6		81.76	
beef burger	400	16.5	(minced meat)	66	
chopped steak	560	5.7		31.92	
beef stew	1,000	9.5		95	
poulet	300	2		6	
rib steak / stockade	500	9.5		47.5	
beef shank	300	3		9	
sum	5,120			584.68	11.42

Ready-made package

	grams of meat	% of fat, unprepared (NEVO)		grams of meat	% of fat (average)
Minced meat	2,000	16.5		330	
Beef burger	1,000	16.5		165	
chopped steak	1,000	5.7		57	
Sausage	1,000	14.6		146	
Sum	5,000			698	**13.96**