SCIENTIFIC COMMITTEE established at the Federal Agency for the Safety of the Food Chain

Salicylic acid residues in products of animal origin

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Declarations of interest: The declarations of interest of any expert contributing to this publication can be requested by contacting Secretariaat.SciCom@favv-afsca.be. Eventual interests on the dossier are reported at the end of the opinion.

Acknowledgement: In addition to the acknowledgements already present in the original opinion, Dr. Pascal Richez (TransPharm) is thanked for his contribution.

Suggested citation: Houben, K., Delahaut, P., Scippo, M.-L., Daeseleire, E., Croubels, S., Urbain, B., Vandenplas, Y., Verheggen, F., Gillard, N. (2024). Salicylic acid residues in products of animal origin. *Food Risk Assess Europe. Volume 2 issue 1 2024 (28 pp) FR-0015 https://doi.org/10.2903/fr.efsa.2024.FR-0015*

Abstract:

The presence of salicylic acid residues was repeatedly detected in milk samples sampled by the Federal Agency for the Safety of the Food Chain (FASFC) and the sectors MelkBe, FEBEV and Algemeen Boerensyndicaat. However, in most cases, investigations by the FASFC's Local Control Units could not identify medical treatment or the use of a biocide that could be linked to the presence of these residues. The sectors therefore question the possibility of

an origin other than medical treatment of the animals or the use of biocides, in particular through plants consumed by grazing animals or supplementary feed. The Scientific Committee investigated this hypothesis on the basis of expert opinion, data from control plans (FASFC monitoring 2017-2021 and Member State reports between 2018 and 2020) or sectoral plans, and a study of scientific literature.

Salicylic acid is a pharmacologically active substance authorised as a veterinary medicine, but is also present in biocidal products authorised in Belgium. Exceedances of maximum residue limits (MRLs) resulting from the administration of veterinary medicines or the application of biocides cannot be ruled out, but would rather be linked to incorrect use of veterinary medicines (incorrect dose administered, non-compliance with the withdrawal period or target species, etc.) or biocides (failure to rinse or incomplete rinsing, use of unauthorised biocides for milking installations, accumulation of residues due to simultaneous use of several biocides containing salicylic acid in the same period, etc.). However, it should be pointed out here that the MRL defined for milk is extremely low and that this value is the subject of discussion within the network of European Reference Laboratories. Salicylic acid is also a natural component of plants. Amongst feed crops, alfalfa hay (485 mg/kg), clover hay (32 mg/kg) and maize (up to 12.8 mg/kg) have been identified as potentially important sources of salicylic acid in cattle. Amongst wild species, willow bark (up to 3000 mg/kg) is also an important source. The listed salicylic acid concentrations in these plant sources should be interpreted with caution, as they may vary considerably from variety to variety, and depend on the part of the plant involved (leaves, flowers, seeds), stress or geographical origin and growing conditions. With regard to possible transfer of salicylates ingested through feed to animal tissues, relatively few studies are available. Although studies have been conducted for ruminants in the context of veterinary drug registration, there are no studies that have investigated transfer from animal feed. It is important to note that in polygastric animals such as dairy cows, salicylic acid is mainly retained in the rumen by ion trapping and transfer to plasma is therefore very limited. This particularity of ruminants makes it possible to reduce absorption by a factor of 1000 and therefore reduces the concentrations present in the tissues and in the milk. On the basis of a daily ration in accordance with good agronomic practice, it was estimated that an adult bovine could consume about 2 g of salicylic acid per day through its feed. Based on the available information, these amounts are not expected to lead to the MRL being exceeded. When ingested at high doses (> 700 mg/litre plasma), salicylates can cause severe toxicity to human health. However, the levels found in plants and feed do not represent a risk to human or animal health. In the opinion of the Scientific Committee and according to the current state of knowledge, the presence of salicylic acid residues in milk following the consumption of plants containing a high level of salicylic acid is unlikely, even in cases of consumption of plant materials very rich in salicylic acid. Salicylates hydrolyse to salicylic acid in vivo. Consequently, it is impossible to determine whether salicylic acid detected in a sample was originally salicylic acid, acetylsalicylic acid, methylsalicylic acid or another salicylate. For the same reason, it is currently impossible to distinguish in ruminants between salicylic acid of feed (natural) or non-feed origin (drug treatment or biocide residues). For the Scientific Committee, it nevertheless is logical to examine the non-feed origin first, given the concentrations that can be achieved in certain biocides (0.1 to 0.5%) or drugs (660 mg/g methyl salicylate) in comparison with the concentrations present in animal feed.

In cases of non-conformities and in addition to investigations into the improper use of drugs or biocides, the Scientific Committee recommends also checking whether an alfalfa-rich

feed was given to the animals and whether willow-based feed supplements were used. In addition, due to the limited number of studies found on the salicylic acid content in alfalfa and due to the importance of alfalfa in animal feed, it may be recommended to conduct further studies on the salicylic acid content of this plant source. In addition, the use of biocides and compliance with their conditions of use should also be monitored. Ideally, warnings about the use of salicylic acid should be included in the technical data sheets or authorisation documents of biocidal products, pointing out the possibility of it being found in milk or meat. In order to establish the possible transfer rates from feed to animal products, further studies should be carried out, especially in ruminants. Furthermore, the Scientific Committee recommends the development of analytical tools allowing to distinguish between a feed (natural) origin and an exogenous origin (medical treatment or contamination with biocides) of salicylic acid residues in animal tissues and more specifically in milk. In view of the recent detections, the Scientific Committee recommends that trends in the detection of salicylic acid in bovine milk in the coming years be analysed and that epidemiological studies be carried out as thoroughly as possible into the potential causes of MRL exceedance.

Keywords: salicylic acid, products of animal origin, milk, meat, cattle, biocides, plants, drug residues

SciCom

OPINION 01-2023

Regarding:

Salicylic acid residues in products of animal origin

(SciCom 2022/12)

Scientific opinion approved by the Scientific Committee on the 30th of January 2023

Keywords:
salicylic acid, products of animal origin, milk, meat, cattle, biocides, plants, drug residues

Contents

Summary

Salicylic acid residues in products of animal origin

Background & Terms of reference

The presence of salicylic acid residues was repeatedly detected in milk samples sampled by the FASFC and the sectors MelkBe, FEBEV and Algemeen Boerensyndicaat. However, in most cases, investigations by the FASFC's Local Control Units could not identify medical treatment or the use of a biocide that could be linked to the presence of these residues. The sectors therefore question the possibility of an origin other than medical treatment of the animals or the use of biocides, in particular through plants consumed by grazing animals or supplementary feed.

For these reasons, the Scientific Committee is asked to provide an opinion on the occurrence of salicylic acid residues in products of animal origin. In particular, it aims to answer the following questions:

- What are the possible natural sources of salicylic acid residues in products of animal origin, and particularly in milk and muscle?
- Is it possible to distinguish between possible natural sources and exogenous administration of salicylic acid?
- What are the animal health and food safety risks associated with the presence of salicylic acid residues in products of animal origin, and can management options be recommended to reduce the risk of occurrence of such residues?

Method

The advice is based on expert opinion, on data from control plans (FASFC monitoring 2017-2021 and Member State reports between 2018 and 2020) or sectoral plans and on a study of scientific literature.

Conclusions

Salicylic acid is a pharmacologically active substance authorised as a veterinary medicine, but is also present in biocidal products authorised in Belgium. Exceedances of maximum residue limits (MRLs) resulting from the administration of veterinary medicines or the application of biocides cannot be ruled out, but would rather be linked to incorrect use of veterinary medicines (incorrect dose administered, non-compliance with the withdrawal period or target species, etc.) or biocides (failure to rinse or incomplete rinsing, use of unauthorised biocides for milking installations, accumulation of residues due to simultaneous use of several biocides containing salicylic acid in the same period, etc.).

However, it should be pointed out here that the MRL defined for milk is extremely low and that this value is the subject of discussion within the network of European Reference Laboratories.

Salicylic acid is also a natural component of plants. Amongst feed crops, alfalfa hay (485 mg/kg), clover hay (32 mg/kg) and maize (up to 12.8 mg/kg) have been identified as potentially important sources of salicylic acid in cattle. Amongst wild species, willow bark (up to 3000 mg/kg) is also an important source. The listed salicylic acid concentrations in these plant sources should be interpreted with caution, as they may vary considerably from variety to variety, and depend on the part of the plant involved (leaves, flowers, seeds), stress or geographical origin and growing conditions.

With regard to possible transfer of salicylates ingested through feed to animal tissues, relatively few studies are available. Although studies have been conducted for ruminants in the context of veterinary drug registration, there are no studies that have investigated transfer from animal feed. It is important to note that in polygastric animals such as dairy cows, salicylic acid is mainly retained in the rumen by ion trapping and transfer to plasma is therefore very limited. This particularity of ruminants makes it possible to reduce absorption by a factor of 1000 and therefore reduces the concentrations present in the tissues and in the milk.

On the basis of a daily ration in accordance with good agronomic practice, it was estimated that an adult bovine could consume about 2 g of salicylic acid per day through its feed. Based on the available information, these amounts are not expected to lead to the MRL being exceeded.

When ingested at high doses (> 700 mg/litre plasma), salicylates can cause severe toxicity to human health. However, the levels found in plants and feed do not represent a risk to human or animal health.

In the opinion of the Scientific Committee and according to the current state of knowledge, the presence of salicylic acid residues in milk following the consumption of plants containing a high level of salicylic acid is unlikely, even in cases of consumption of plant materials very rich in salicylic acid.

Salicylates hydrolyse to salicylic acid *in vivo*. Consequently, it is impossible to determine whether salicylic acid detected in a sample was originally salicylic acid, acetylsalicylic acid, methylsalicylic acid or another salicylate. For the same reason, it is currently impossible to distinguish in ruminants between salicylic acid of feed (natural) or non-feed origin (drug treatment or biocide residues). For the Scientific Committee, it nevertheless is logical to examine the non-feed origin first, given the concentrations that can be achieved in certain biocides (0.1 to 0.5%) or drugs (660 mg/g methyl salicylate) in comparison with the concentrations present in animal feed.

Recommandations

To the authorities

In cases of non-conformities and in addition to investigations into the improper use of drugs or biocides, the Scientific Committee recommends also checking whether an alfalfa-rich feed was given to the animals and whether willow-based feed supplements were used. In addition, due to the limited number of studies found on the salicylic acid content in alfalfa and due to the importance of alfalfa in animal feed, it may be recommended to conduct further studies on the salicylic acid content of this plant source.

In addition, the use of biocides and compliance with their conditions of use should also be monitored. Ideally, warnings about the use of salicylic acid should be included in the technical data sheets or authorisation documents of biocidal products, pointing out the possibility of it being found in milk or meat.

In order to establish the possible transfer rates from feed to animal products, further studies should be carried out, especially in ruminants.

Furthermore, the Scientific Committee recommends the development of analytical tools allowing to distinguish between a feed (natural) origin and an exogenous origin (medical treatment or contamination with biocides) of salicylic acid residues in animal tissues and more specifically in milk.

In view of the recent detections, the Scientific Committee recommends that trends in the detection of salicylic acid in bovine milk in the coming years be analysed and that epidemiological studies be carried out as thoroughly as possible into the potential causes of MRL exceedance.

1. Terms of reference

1.1. Questions

The Scientific Committee is requested to issue an opinion on the presence of salicylic acid residues in products of animal origin. In particular, the objective is to answer the following questions:

- What are the possible natural sources of salicylic acid residues in products of animal origin, and particularly in milk and muscle?
- Is it possible to distinguish between possible natural sources and exogenous administration of salicylic acid?
- What are the animal health and food safety risks associated with the presence of salicylic acid residues in products of animal origin, and can management options be recommended to reduce the risk of occurrence of such residues?

For this opinion, the Scientific Committee interpreted the question as it was received by the FASFC administration as follows: for salicylic acid residues in products of animal origin, is there any other possible origin than the medicinal treatment of animals or the use of biocides, in particular a natural origin through plants consumed by grazing animals or complementary feed?

1.2. Legal provisions

Commission Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin.

Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products.

1.3. Method

The advice is based on expert opinion, on data from control plans (FASFC monitoring 2017-2021 and Member State reports between 2018 and 2020) or sectoral plans and on a study of scientific literature.

2. Definitions & Abbreviations

ADI	Acceptable Daily Intake
NOAEL	*No Observable Adverse Effect Level*
BVL	*Bundesamt für Verbraucherschutz und Lebensmittelsicherheit*, European Reference Laboratory for the detection of salicylic acid (Germany)
CCα	Decision limit; limit at and above which it is permissible to conclude with an alpha probability of error that a sample is non-compliant (source: FASFC document "Inventaris acties en actiegrenzen en voorstellen voor harmonisering in het kader van de officiële controles - Chemische contaminanten, residuenen additieven").
Cl	*Total body clearance*, time for total clearance from the body
EMA	European Medicines Agency
EMEA	European Agency for the Evaluation of Medicinal Products
IRMS	Isotope ratio mass spectrometry
MRL	Maximum residue limit
NSAIDs	Nonsteroidal anti-inflammatory drugs
$T_{1/2el}$	Elimination half-life
Vd	Volume of distribution at steady state

Considering the discussions during the working group meetings on 5 September 2022 and 6 October 2022, along with the plenary sessions of the Scientific Committee on 16 December 2022 and 20 January 2023,

the Scientific Committee provides the following opinion:

3. Introduction/context

Salicylic acid (C7H6O3, 2-hydroxybenzoic acid) is an aromatic organic compound found naturally in certain plants (Keszycka *et al.*, 2017). Its medicinal properties have been known since ancient times, when it was extracted from willow for its antipyretic/anti-inflammatory properties. Although salicylic acid has since been supplanted in humans by other, more effective substances, such as acetylsalicylic acid and paracetamol, for the treatment of pain and fever, it is currently widely used in dermatology as a complement to other active ingredients.

Salicylic acid is authorised as a veterinary medicinal product for food-producing animals (Regulation (EU) n°37/2010). This authorisation covers several pharmacologically active substances, and maximum residue limits (MRLs) are set for many of them. Salicylic acid is also a component of several biocidal products authorised in Belgium and subject to the European biocides legislation (Regulation (EU) No. 528/2012).

Salicylic acid and its derivatives occur naturally in plants such as willow, meadowsweet, rice, corn, soybeans and barley, and grasses such as *Festulolium sp.* (a hybrid between different pasture grass genera) and European orchardgrass (Raskin *et al.*, 1990; Harbourne *et al.*, 2009; Pociecha *et al.*, 2009). It is a phytohormone involved in the activation of plant defence mechanisms against abiotic and biotic stresses (Fragnière *et al.*, 2011). Exogenous applications of salicylic acid can also improve plant resistance to certain environmental stresses. The latter is described for maize, for example, in studies by Purcarea and Cachita-Cosma (2010), El-Katony *et al.* (2019), and Shemi *et al.* (2021).

Salicylic acid residues have been detected on several occasions in milk samples taken by the FASFC and by the MelkBe, FEBEV and *Algemeen Boerensyndicaat* sectors. In most cases, investigations by the FASFC's local control units failed to identify any medicinal treatment or biocide use that could explain the presence of these residues.

4. Advice

4.1. Non-compliances concerning salicylic acid residues in milk and meat

Salicylic acid residues have been detected on several occasions in milk samples taken and analysed by the FASFC and the MilkBE, FEBEV and *Algemeen Boerensyndicaat* sectors. As part of the 2017-2021 FASFC monitoring, nine milk samples (including five in 2021) contained salicylic acid residues in concentrations above the method's CCα (9.60 µg/kg) and were therefore considered non-compliant. All the milk samples were taken from the milk tank. Salicylic acid residues were recently identified in a bovine muscle sample, again without any evidence of non-steroidal anti-inflammatory drugs or accidental contamination.

As part of the Monimilk sector monitoring, ten milk samples have been analysed every year since 2014 for the presence of NSAID residues, including salicylic acid. In 2018, salicylic acid was detected in a milk sample at a concentration of 10.8 µg/kg, above the MRL but below the CCα (CCα at 12 µg/kg); this

sample was therefore compliant. In 2021, salicylic acid was detected in a milk sample at a concentration of 11.6 µg/kg (CCα at 9.9 µg/kg); this sample was therefore non-compliant. The differences in CCα are due to the different methods used by different laboratories.

On a European scale and based on information provided by the European Reference Laboratory (EURL-BVL), five Member States reported non-compliant results for the presence of salicylic acid in milk over the 2018-2020 period (seven non-compliant results out of 995 samples analysed). However, no information is available on the origin of the residues. It should also be stressed that salicylic acid testing is only considered "optional" by the EURL and the European Commission as part of veterinary drug residue monitoring. Salicylic acid is therefore not systematically analysed.

4.2. Veterinary medicines and biocidal products containing salicylic acid

Salicylic acid residues detected in products of animal origin, such as milk and meat, may come from biocides and/or authorised veterinary medicines containing salicylic acid or its precursors.

Veterinary drugs

Regulation (EU) no. 37/2010 lists all the pharmacologically active substances authorised in Europe, many of which contain salicylic acid as the parent substance, in the form of salts or precursors (acetylsalicylic acid, DL-lysine acetylsalicylic acid, basic aluminium salicylate, bismuth subsalicylate, hydroxyethyl salicylate, methyl salicylate, sodium acetylsalicylate and sodium salicylate). When a marker residue is specified for these substances, it is salicylic acid, i.e. the same residue, salicylic acid, is used to detect all these substances. **Table 1** below shows an extract of Regulation 37/2010 listing the MRLs for pharmacologically active substances for which salicylic acid is the marker residue.

Salicylic acid (as a parent substance) is authorised for topical use only for all food-producing species with the exception of fish. Therefore, no MRL is set for this topical use. Basic aluminium salicylate is approved for *per os* or topical use. When used orally in cattle, goats, rabbits and horses, MRLs are set for tissues (e.g. 200 µg/kg in muscle) and milk (9 µg/kg). No MRL is required when used topically in all food-producing species except cattle, goats, equines, rabbits and fish. Sodium salicylate is also approved for *per os* or topical use. Its use in cattle and pigs is limited to the oral route and the substance cannot be used in milk-producing animals. It is authorised for topical use for all food-producing species with the exception of fish. No MRL is required in either case. Tissue MRLs have been set (e.g. 400 µg/kg in muscle) when sodium salicylate is used as an anti-inflammatory agent/NSAID in turkey.

There is therefore clearly an ambiguity concerning MRLs and authorisation for its use depending on the treatment route. It should be noted that some topical formulations can contain up to 660 mg/g of methyl salicylate.

It is also important to remember that in livestock, for example pigs and cattle, salicylates can be used as a first-line treatment in the event of fever, with the aim of reducing antibiotic use. No MRLs should be exceeded if salicylates are used in compliance with the recommended doses and required withdrawal periods.

Biocides

Salicylic acid is also present in many biocides authorised in Belgium. Mainly for cleaning and disinfecting the teats of dairy cows, but also for disinfecting milking installations and treating animal bedding. **Appendix 1** gives an overview of the biocides authorised in Belgium by the FPS Public Health, Food Chain Safety and Environment as of 9 September 2022. It should be noted that most biocides, including those intended for direct application to the teats of dairy cattle, contain salicylic acid levels ranging from 0.1 to 0.5%. Two products contain higher concentrations of salicylic acid (2.5-15%), but these products are either not intended for direct application to humans or animals (surface

disinfection), or are not authorised for use on milking equipment. It should also be noted that the biocides list also contains products to which salicylic acid is added not for its biocidal action but as a stabiliser, and at lower concentrations (0.05-0.26%).

When registering a biocide, one of the mandatory data items is the substance's MRL (Regulation (EU) No. 528/2012). Each manufacturer must also specify the conditions of use (teat cup rinsing, udder cleaning, etc.). This means that, as with veterinary medicines, the MRL of an active substance must also be respected for biocidal products. When biocides are used in accordance with best practice (rinsing, time of use, etc.), the risk of transfer to products of animal origin is limited and should not lead to MRLs being exceeded.

A recent study by Fitzpatrick *et al*. (2021) describes the effectiveness of the biocides used in Ireland to control the bacteria associated with mastitis in dairy cows. Ten disinfectants containing a combination of lactic acid and salicylic acid (max. 3% concentration) were applied to the teats of Holstein-Frisonne cows, leading to a reduction in the bacteria present. However, this study did not test the presence of salicylic acid residues in milk.

The MRL values for drugs are determined by the European Medicines Agency (EMA) on the basis of residue depletion studies in which the drugs are administered to animals according to a recommended therapeutic regimen. Biocide authorisation studies include a risk assessment of the active substances concerned and the recommended doses of biocides on animals should be safe if used correctly.

The exceeding of MRLs due to the administration of veterinary drugs or biocides cannot be ruled out but would be linked to the incorrect use of veterinary drugs (incorrect dose administered, failure to observe the withdrawal period or target species, etc.) or biocides (lack of rinsing or incomplete rinsing, use of biocides not authorised for milking facilities, accumulation of residues following the simultaneous use of several biocides containing salicylic acid over the same period, etc.).

Table 1. Extract of Regulation (EU) no. 37/2010 listing the maximum residue limits (MRLs) for pharmacologically active substances

Pharmacologically active substance	Marker residue	Animal species	MRL	Targeted tissues	Other provisions [according to Article 14(7) of Regulation (EC) No 470/2009]	Therapeutic classification
Salicylic acid	NOT APPLICABLE	All food-producing species except fin fish	No MRL required	NOT APPLICABLE	For topical use only	NO ENTRY
Aluminium salicylate, basic	Salicylic acid	Bovine, caprine, *Equidae*, rabbit	200 µg/kg 500 µg/kg 1,500 µg/kg 1,500 µg/kg	Muscles, Fat Liver, Kidney	NO ENTRY	Antidiarrheal and intestinal anti-inflammatory agents
		Bovine, caprine, *Equidae*	9 µg/kg	Milk		
	NOT APPLICABLE	All food-producing species with the exception of cattle, goats, equines, rabbits and fish	No MRL required	NOT APPLICABLE	For topical use only	
Methyl salicylate	NOT APPLICABLE	All food-producing species except fin fish	No MRL required	NOT APPLICABLE	For topical use only	NO ENTRY
Sodium salicylate	NOT APPLICABLE	Bovine, porcine	No MRL required	NOT APPLICABLE	For oral use. Not for use in animals producing milk for human consumption.	NO ENTRY
		All food-producing species except fin fish	No MRL required	NOT APPLICABLE	For topical use only	
	Salicylic acid	Turkeys	400 µg/kg 2,500µg/kg 200 µg/kg	Muscle, Skin and fat in natural	Not for use in animals producing eggs for human consumption	Anti-inflammatory agents/non-steroidal anti-inflammatory agents

			150 µg/kg	proportions, Liver, Kidney		

4.3. Natural sources of salicylic acid

A review of the literature on the natural presence of salicylic acid in plants was carried out. It focused on salicylic acid levels in herbaceous plants, feeds and animal feed supplements, as presented in **Appendix 2**. Salicylic acid levels in other plant matrices or foodstuffs are beyond the scope of this study and are not discussed here.

Several studies have demonstrated the presence of salicylic acid in herbaceous plants suitable for consumption by livestock, in a range from 0.01 to 5 mg/kg. The reported concentrations are 5 mg/kg for crabgrass leaves, 3.5 mg/kg for green foxtail leaves (Raskin *et al.*, 1990) and 1.5 mg/kg for *Festulolium sp.* (Pociecha *et al.*, 2009) and 0.95 mg/kg for meadowsweet flowers (Fecka, 2009).

Other plants are inexpensive sources of protein, making them interesting to feed to livestock. Some of these plants (alfalfa, clover and timothy) may also contain high concentrations of salicylic acid. Beaumier *et al.* (1987) measured salicylic acid concentrations of 485 mg/kg in alfalfa hay and pressed alfalfa hay, 32 mg/kg in clover hay and 2.4 mg/kg in timothy hay. The study by Iqbal *et al.* (2021) also determined a relatively high salicylic acid content for alfalfa, i.e. 1,440.64 ± 0.04 mg/kg dry weight for the vegetative parts (leaves and stem) of alfalfa.

Willow is also a well-known source of salicylic acid. Species of the genus naturally contain high levels of salicylic acid, with concentrations of up to 2,200 mg/kg in the branches and 3,000 mg/kg in the bark (Petrek *et al.*, 2007). It is not inconceivable that livestock, especially cattle grazing in willow-lined pastures, could eat pieces of bark and so absorb salicylic acid. On the other hand, willow bark is used in certain supplements for its properties associated with salicylic acid. As far as willow branches are concerned, cows have no incisors in their upper jaw. As a result, they are unable to tear off branches.

Protasiuk and Olejnik (2018) and Keszycka *et al.* (2017) determined the salicylic acid content of compound feed and their raw materials for laying hens, pigs and cattle, as well as alternative supplements or components (see **Appendix 2**).

In compound animal feed, the salicylic acid concentrations detected ranged from less than 0.05 mg/kg to 0.48 mg/kg. In raw materials, rather negligible levels of salicylic acid were measured in cereals, with the exception of roasted buckwheat (14.21 mg/kg), while higher concentrations were measured in paprika powder (up to 1.87 mg/kg) and corn (1.01 mg/kg for mature corn, 12.8 mg/kg for young corn, 2.28 mg/kg for cornflower).

Several studies have also identified vegetables and fruits as a source of salicylic acid (Robertson and Kermode, 1981; Scotter *et al.*, 2007; Keszycka *et al.*, 2017; Protasiuk and Olejnik, 2018): soya (1.18 for leaves and 2.01 mg/kg for flowers), fresh butter beans (1.31 mg/kg), fresh cauliflower (5.44 mg/kg), fresh celery (2.85 mg/kg), raw lentil seeds (16.76 mg/kg), raw pea seeds (1.44 mg/kg), cooked potatoes (1.20 mg/kg), peanuts (0.22 mg/kg), fresh tomatoes (1.3 mg/kg), plums (1.77 mg/kg), strawberries (2.25 mg/kg) and watermelon (2.67 mg/kg). Although less relevant in the context of animal feed, it can be deduced from these studies that salicylic acid content decreases with heat treatment (cooking) and fruit ripening.

The above discussion clearly demonstrates the presence of salicylic acid in many plants but shows that salicylic acid contents can vary considerably from one variety to another and depend on the part of the plant concerned (leaves, flowers, seeds). Moreover, concentrations can increase under (a)biotic stress and vary according to plant genotype, geographical origin and growing conditions.

If we consider the daily rations of cattle and their consumption of alfalfa in compliance with good agronomic practices (4-5 kg/day) and corn (18 kg of corn for the whole plant/corn silage or 12 kg of corn grain), an adult bovine could consume around 2,000 mg of salicylic acid per day through its diet.

For horses, the study by Beaumier *et al.* (1987) assessed that the maximum daily load of salicylate from their feed should not exceed 2 g given their feed composition (Keszycka *et al.*, 2017). It should be noted that the digestive physiology of horses (monogastrics) is different from that of cattle (ruminants). The pH is around 5 at the start of digestion, falling to 2 at the end under the action of the gastric juices.

4.4. Salicylic acid content of products of animal origin

A number of studies have been carried out to assess salicylic acid levels in products of animal origin. Keszycka *et al.* (2017) demonstrated the presence of salicylic acid in marketed foods such as meat, fish, eggs, milk and dairy products (**Appendix 3)**. Other studies, however, found no salicylic acid or acetylsalicylic acid residues in pork muscle, milk, shrimp, eel and flatfish sampled in South Korea (Zheng *et al.*, 2019). Lastly, cases of salicylic acid residues in milk have also been reported in several EU Member States by the European Reference Laboratory EURL-BVL (*Bundesamt für Verbraucherschutz und Lebensmittelsicherheit*). This study showed that the highest salicylic acid concentrations were recorded in Croatia and Hungary in particular, but it was unable to identify the cause of these regional differences.

However, these studies did not identify the cause of the presence of residues, which could be related to the administration of drugs to animals, the use of biocides or the consumption of feed containing salicylates.

The EMA's public reports detail the studies carried out during the registration of veterinary medicinal products, and thus the concentrations of marker residues detected after administration, the withdrawal times and the MRLs set for each target species. Report EMA/CVMP/454104/2016 of 12-12-2016 mentions residue depletion studies for calves and adult dairy cows. In the first study, eight calves (four males, four females), with an average weight of 53 kg, received 400 mg basic aluminium salicylate per kg of body weight/day for five consecutive days, administered twice daily. The average salicylic acid concentrations 24 hours after administration were 871 µg/kg in muscle, 1,886 µg/kg in liver, 6,702 µg/kg in kidney and 1,289 µg/kg in fat. Concentrations three days after administration were below the quantification limit in muscle and were 1,203 µg/kg in liver, 646 µg/kg in kidney and 236 µg/kg in fat. Salicylic acid depletion was therefore rapid, with slower elimination in the liver than the other tissues.

In the second EMA study (EMA/CVMP/454104/2016), the highest recommended therapeutic dose in adult cattle (9 g of basic aluminium salicylate per animal) was administered orally to 10 Holstein-Frisonne cows three times a day, for three consecutive days. This dose corresponds to approximately 43.5 mg salicylic acid equivalent/kg body weight/day. The study included five low-milk-yield cows (less than 10.5 kg milk per day) and five high-milk-yield cows (more than 10.5 kg milk per day). All salicylic acid concentrations but one (178 µg/kg, during treatment) were below 110 µg/kg. The average concentrations were 89 µg/kg one hour before the last administration, decreasing to 34, 14, 5 and 5 µg/kg, 12, 24, 36 and 48 hours respectively after the last administration. From this data, it can be deduced that exceeding the MRL of 9 µg/kg in milk is not inconceivable if withdrawal times after the administration of salicylates as veterinary drugs are not respected, or if (unintended) excessive doses of these substances were ingested.

For the sake of completeness, we should also mention the EMEA/MRL/695/99-FINAL report of November 1999, which mentions residue depletion studies in pigs, poultry and cattle, the

EMEA/MRL/897/04-FINAL report of 2004 on cattle and pigs and the EMA/CVMP/71291/2012 report of January 2013 on turkeys.

Regarding the presence of salicylic acid in animal tissues and its possible origin in animal feed, relatively few studies are available. Protasiuk and Olejnik (2020) studied the residues of salicylic acid and its metabolites in the plasma, tissues and eggs of laying hens following either the medicinal treatment of the animals or the consumption of naturally occurring salicylates via the feed. In the first case, hens were given sodium salicylate or acetylsalicylic acid via drinking water (10 mg/kg body weight) for seven days. The salicylic acid concentrations in the liver were higher than in muscle, with a maximum of 207 µg/kg after the administration of sodium salicylate. In eggs, the plateau concentration was a maximum of 275 µg/kg. Gentisic acid was also found in liver, muscle and egg samples. In the second case, the animals were fed fresh corn naturally containing 1.18 mg/kg salicylic acid. After this administration, salicylic acid was found only in negligible amounts in liver, muscle, plasma and eggs, with a maximum of 9.72 µg/kg in muscle and 82.3 µg/kg in eggs. Beaumier *et al.* (1987) studied the presence of salicylic acid in the urine and plasma of Standardbred horses fed a twice-daily diet rich in alfalfa. Given the composition of the animal feed in this study, the maximum daily intake did not exceed 2 g of salicylates. Increased salicylic acid levels were observed in equine urine and plasma, with maximum salicylic acid concentrations of 363 µg/ml in urine and 3.6 µg/ml in plasma.

The studies by Protasiuk and Olejnik (2020) and Beaumier *et al.* (1987) involved monogastric animals. No similar studies have been carried out on ruminants to demonstrate a transfer of salicylic acid from feed to animal products such as milk and meat.

Salicylic acid and other salicylates are metabolised as a glycine conjugate before being excreted by the kidneys. The resulting compound is known as salicyluric acid. A recent study by Bongers *et al.* (2022) revealed that salicyluric acid was present in milk. The storage of milk at room temperature leads to the hydrolysis of salicyluric acid, releasing salicylic acid at concentrations that may exceed the MRL (9 µg/kg), as well as the para-isomer 4-hydroxybenzoic acid. While the identity of the compounds studied was confirmed by high-resolution mass spectrometry (HRMS), it was also demonstrated that a decrease of one mole of salicyluric acid does not seem to result in a one-mole increase in salicylic acid; the formation of salicylic acid in milk can therefore only be partially attributed to the hydrolysis of salicyluric acid. It should also be noted that to avoid the appearance of salicylic acid during storage, the milk can be stabilised by adding an organic solvent (10% v/v methanol) or lowering the pH (10% formic acid or 0.5% acetic acid) as soon as it is received by a laboratory.

Only the study by Bongers *et al.* (2022) reported the formation of salicylic acid in milk during storage at room temperature. It would be desirable to confirm this hypothesis with further trials. Consequently, the study by Bongers et al (2022) is included in the Uncertainties section of this opinion (see Section 5).

4.5. Metabolism data and distinction between possible sources of salicylic acid

4.5.1. Absorption: the specific case of ruminants

Most drugs are weak organic bases or acids and exist in solution in both non-ionised and ionised forms. According to the pH distribution hypothesis, the non-ionised form is generally fat-soluble and can easily diffuse across the cell membrane to reach the same equilibrium concentration on both sides. In contrast, the ionised form is often virtually excluded from transmembrane diffusion due to its low lipid solubility (Booth and McDonald, 1991).

Salicylic acid is a weak organic acid. The ratio between the non-ionised and ionised forms of a drug is expressed by the Henderson-Hasselbalch equation. The Henderson-Hasselbalch equation for an acid is given in **Equation 1**.

$$pH = pKa - log\frac{(ionised\ conc.)}{(non-\text{ionised}conc.)}$$

Equation 1. Henderson-Hasselbalch equation for an acid

In ruminants, the rumen is very important for digestion. The rumen has a higher pH, compared with the human stomach (pH = 1.4) for example. Rumen pH is around 6.4. Assuming the pH (= 6.4) of the rumen, a pH of 7.4 for plasma and the pKa (= 3.4) of salicylic acid, and assuming that the non-ionised form is in equilibrium (non-ionised conc. = 1), we obtain a difference of 1,000 between the concentration of ionised salicylic acid and that of non-ionised salicylic acid in the rumen. Most of the salicylic acid therefore remains in the rumen, with little or no transfer to the plasma. This phenomenon is known as ion trapping. In dairy cows, this means that the percentage of salicylic acid that could pass from the rumen to the milk is very limited (reduced by a factor of 1,000).

It should be noted, however, that cows may suffer from subacute rumen acidosis (ruminal acidosis), which could increase salicylic acid absorption. This condition occurs when rumen pH falls below 5.8, and can be relatively common in high-producing dairy cows. However, acidosis is often linked to a high consumption of highly fermentable plant sources, such as cereals, which are therefore not significant sources of salicylic acid.

4.5.2. Salicylic acid metabolism

In vivo, acetylsalicylic acid and methylsalicylic acid are rapidly hydrolysed to salicylic acid. This process has been widely described in humans and, to a lesser extent, in other mammals (Pozniak *et al.*, 2013). As acetylsalicylic acid is already converted to salicylic acid in the gastrointestinal tract, salicylic acid and acetylsalicylic acid have similar metabolic pathways (Protasiuk and Olejnik, 2018). Once absorbed, acetylsalicylic acid is rapidly hydrolysed to salicylic acid by the non-specific esterases present in the intestinal wall, liver and red blood cells. While some salicylic acid is subsequently eliminated unchanged, variable amounts are conjugated with glycine to produce salicyluric acid, and with glucuronic acid to form salicylphenol glucuronide, the main urinary metabolites of acetylsalicylic acid. In addition, salicylacyl glucuronide and the hydroxylation products of the salicylic acid cycle - gentisic acid and the glycine-conjugation product of gentisic acid - are also formed and excreted in the urine in small quantities. The relative proportions of metabolites vary according to the salicylate concentrations to be eliminated (Ciejka *et al.*, 2016; Vale, 2016). It should be noted that metabolism differs slightly in some birds, including hens; conjugation with glycine does not take place and is replaced by the conjugation of salicylic acid with ornithine (Baert *et al.*, 2004).

Table 2 below shows the pharmacokinetic variables for the metabolism of acetylsalicylic acid by chickens and salicylic acid by chickens and cattle (Coetzee *et al.*, 2007; Pozniak *et al.*, 2013). In general, the half-life increases with low urinary pH and poor renal function. To our knowledge, these values are not available for pigs.

Table 2. Pharmacokinetic variables for the metabolism of acetylsalicylic acid by chickens and salicylic acid by chickens and cattle

Acetylsalicylic acid	Hens*	
$T_{1/2el}$	0.25-0.4 h	
Cl	3.35 l/h·kg	
Vd	0.8 l/kg	
Salicylic acid	Hens*	Cattle**
$T_{1/2el}$	3-4 h	0.5-0.75 h
Cl	0.05-0.2 l/h·kg	0.2-0.3 l/h·kg

Vd	/	0.2 l/kg

* Source: Pozniak *et al.* (2013)

** Source: Coetzee *et al.* (2007)

$T_{1/2el}$ = elimination half-life; Cl = total body clearance; Vd = volume of distribution at steady-state

4.5.3. Distinction between possible sources of salicylic acid

Due to the *in vivo* hydrolysis of salicylates, it is impossible to determine whether the salicylic acid detected in a sample was originally salicylic acid, acetylsalicylic acid, methylsalicylic acid or another salicylate. Just as it is not possible to identify the active substance, it is also not currently possible to use a detection method to distinguish between residues resulting from the use of a veterinary drug (e.g. basic aluminium salicylate), a biocide or the ingestion of plants containing salicylic acid.

Isotope ratio mass spectrometry (IRMS) is a technique that could be used to determine the natural origin or exogenous nature (biocide or veterinary drug) of salicylic acid residues in animal matrices. One example is the study by Krummen *et al.* (2004), in which LC-IRMS was applied to different sources of natural acetylsalicylic acid and paracetamol from different suppliers to combat drug counterfeiting. To date, however, there is no real proof that this strategy would be effective for residue analysis. It should also be noted that IRMS is a cumbersome and costly method, making it difficult to apply routinely.

A second strategy that could be used to distinguish natural sources from the exogenous administration of salicylic acid is the comparison of concentrations of salicylic acid and one or more of its metabolites (e.g. gentisic acid) or the establishment of maximum tolerance levels of salicylic acid. Beaumier *et al.* (1987) studied the ratio between salicylic acid and its metabolite gentisic acid in horse urine. Unfortunately, this ratio proved non-discriminatory and it was therefore necessary to establish maximum tolerance levels for salicylic acid in plasma and urine. Plasma salicylic acid values in excess of 6.5 µg/ml and urinary salicylic acid values in excess of 750 µg/ml could be attributed to drug administration.

Ratios and maximum values must be approached with caution and determined on a case-by-case basis. This requires extensive experimental design involving different combinations of feed, animal species and products of animal origin.

4.6. Determining risks to food safety and animal health

Acetylsalicylic acid is widely used in human medicine as an analgesic, antipyretic, anti-inflammatory, platelet aggregation inhibitor, vasoprotector and antineoplastic agent. It has also been associated with a reduced risk of colorectal and other cancers, including oesophageal, stomach, ovarian, breast and lung cancer (Keszycka *et al.* 2017).

Toxicity can occur following acute ingestion or chronic ingestion leading to high blood levels, and can affect the central nervous system, pulmonary system and gastrointestinal tract. Severe bleeding may occur. Disturbances of acid-base balance are common in cases of acetylsalicylic acid toxicity (American College of Medical Toxicology, 2015).

Salicylate intoxication in adults is associated with the dose of drug ingested and can lead to different clinical features. The clinical features of mild to moderate salicylate intoxication (< 700 mg/litre plasma) include deafness, tinnitus, nausea, vomiting, hyperventilation, sweating, vasodilation, tachycardia, respiratory alkalosis and metabolic acidosis. In cases of severe salicylate intoxication (> 700 mg/litre plasma), the following clinical features can also occur: confusion, delirium, hypotension, cardiac arrest and acidosis. The less common complications of salicylate intoxication include non-

cardiogenic pulmonary oedema, cerebral oedema, convulsions, coma, encephalopathy, renal failure, tetany, hyperpyrexia and hypoglycaemia (Vale, 2016).

Prolonged treatment with low-dose acetylsalicylic acid (< 325 mg per day) is also not without risk. In addition to the increased risk of serious gastrointestinal or cerebral haemorrhage, treatment with low-dose acetylsalicylic acid is associated with a significant increase in the risk of gastrointestinal adverse events. In observational studies, long-term low-dose acetylsalicylic acid has been associated with an increased relative risk of bleeding in the upper and lower gastrointestinal tract (Lavie *et al.*, 2017). Chronic acetylsalicylic acid toxicity is often associated with atypical clinical presentations that may be similar to diabetic ketoacidosis, delirium, stroke, myocardial infarction or heart failure. Salicylate-induced nephrotoxicity increases in later life and the risk of upper gastrointestinal bleeding increases, with a higher mortality rate (Durnas and Cusack, 1992).

Patients treated with moderate- or high-dose acetylsalicylic acid for long periods often have elevated serum alanine aminotransferase levels. Elevations in serum alanine aminotransferases at high doses are frequent and may be clear and accompanied by slight elevations in alkaline phosphatase and bilirubin. The most spectacular examples of acetylsalicylic acid hepatotoxicity usually occur at doses of 1,800 to 3,200 mg per day (> 100 mg/kg) and with salicylate levels above 25 mg/dL in serum, but mild to moderate elevations of serum alanine aminotransferase occur at even lower doses and lower serum values. Acetylsalicylic acid hepatotoxicity is generally mild and asymptomatic, although at higher doses, nausea, anorexia, abdominal pain and even encephalopathy with signs of hepatic dysfunction (hyperammonaemia and coagulopathy) may occur. The increase in bilirubin is generally slight or absent. Mild eosinophilia may accompany enzyme elevation, but rash, fever and other allergic manifestations are rare. Liver biopsy histology usually shows minimal damage despite elevated enzymes; electron microscopy may reveal lipid and mitochondrial abnormalities (*Aspirin*, 2012).

Although teratogenic effects have been observed in animals administered near-lethal doses of acetylsalicylic acid, there is no evidence that this drug is teratogenic in humans. However, acetylsalicylic acid should be avoided during the first and second trimesters of pregnancy, unless absolutely necessary (PubChem CID 2244).

The use of acetylsalicylic acid has been associated with the development of Reye's syndrome in children and teenagers with acute febrile illnesses, particularly influenza and chickenpox. It is characterised by a combination of liver disease and non-inflammatory encephalopathy. However, its association with acetylsalicylic acid use in children is controversial (Casteels-Van Daele, 2000).

In Europe, the EMA has established toxicological threshold values for the veterinary use of salicylates. According to the EMEA/MRL/695/99-FINAL report, the pharmacologically acceptable daily intake (ADI) of acetylsalicylic acid is 0.5 mg/person (0.0083 mg/kg body weight). ADIs were then established for other salicylates for veterinary use, such as sodium salicylate with a pharmacological ADI of 0.5 mg/person (0.0083 mg/kg bw, report EMEA/MRL/897/04-FINAL) or basic aluminium salicylate with an ADI of 0.55 mg/person (0.0091 mg/kg bw, report EMA/CVMP/454104/2016).

It should also be stressed that around 2.5% of Europeans suffer a hypersensitivity to salicylates. The consequences of salicylate hypersensitivity include Widal syndrome, acetylsalicylic acid-induced asthma or asthma with acetylsalicylic acid hypersensitivity, Reye's syndrome (encephalopathy and fatty infiltration of internal organs), angioneurotic oedema and urticaria (Protasiuk and Olejnik, 2018). In addition, acetylsalicylic acid can cause mild haemolysis in people with a glucose-6-phosphate dehydrogenase deficiency (Hardman *et al.*, 2006).

In animal health, Webb and Hansen (1963) studied the chronic and sub-acute toxicology and pathology of methyl salicylate in dogs, rats and rabbits. The effects of chronic exposure to methyl salicylate were

studied in beagles (n=2/sex/group), with weight reduction and liver effects observed at a dose of 150 mg/kg bw/day. Based on this study, Health Canada (Environment and Climate Change Canada, Health Canada, 2020) proposes a NOAEL value of 50 mg/kg bw/day.

Based on the NOAEL of 50 mg/kg bw/day, a dairy cow weighing 500 to 600 kg would need to ingest more than 25 to 30 g of salicylic acid per day for it to present a health risk. However, this is an approximation; the NOAEL has been determined for dogs, and further tests are needed to obtain the correct values for ruminants. The estimated dose of 25 to 30 g of salicylic acid per day is much higher than the dose of 2 g of salicylic acid per day that an adult bovine could absorb through animal feed under good agronomic practice (see Section 4.3). Furthermore, it is assumed that absorption of salicylic acid is lower in ruminants (see Section 4.5.1). It can be concluded that ingesting salicylic acid through feed poses no health risk to ruminants.

5. Uncertainties

The literature review identified plants as potential sources of salicylates. One study evaluated the transfer of these salicylates to animal tissues in chickens. However, no studies have been found on ruminants. The uncertainties in this opinion are therefore linked to a lack of data that could prove whether or not a significant intake of salicylates by cattle via animal feed would lead to the MRLs determined for human health being exceeded in the foodstuffs derived from them.

In milk, it should also be noted that salicylic acid can be formed when milk is stored improperly (at room temperature). This results in part from the hydrolysis of salicyluric acid, a metabolite derived from the conjugation of salicylic acid with glycine (Bongers *et al.*, 2022). As the data comes from a single study, this should be confirmed by further studies, as should possible stabilisation strategies (addition of organic solvent or acidification of the milk).

Two sources of scientific literature were found concerning the salicylic acid content of alfalfa (Beaumier *et al.*, 1987; Iqbal *et al.*, 2021). The relatively high salicylic acid content of alfalfa was confirmed in both sources. Nevertheless, there is a clear difference (around factor 3) between the salicylic acid levels reported in the studies. This could be due, for example, to differences in geographical origin and cultural conditions or to stress, but it is impossible to say for sure. For these reasons, and given the importance of alfalfa in animal feed, further studies on the salicylic acid content of this plant source may be recommended.

6. Conclusions

Salicylic acid is a pharmacologically active substance authorised as a veterinary medicine but also present in biocides authorised in Belgium. The exceeding of maximum residue limits (MRLs) due to the administration of veterinary drugs or the application of biocides cannot be ruled out but would be linked to the incorrect use of veterinary drugs (incorrect dose administered, failure to observe the withdrawal period or target species, etc.) or biocides (lack of rinsing or incomplete rinsing, use of biocides not authorised for milking facilities, accumulation of residues following the simultaneous use of several biocides containing salicylic acid over the same period, etc.).

It should be stressed, however, that the MRL defined for milk is extremely low and that this value is the subject of discussion within the network of European Reference Laboratories.

Salicylic acid is also a compound naturally present in plants. Salicylic acid is produced endogenously during stress and can also be provided exogenously in agronomy to help plants cope with this type of stress. A review of the literature, focusing on salicylic acid levels in herbaceous plants, animal feeds

and feed supplements, identified alfalfa hay (485 mg/kg), clover hay (32 mg/kg) and corn (up to 12.8 mg/kg) among feed crops, and willow bark (up to 3,000 mg/kg) among wild species, as potentially significant sources of salicylic acid in livestock. The salicylic acid concentrations listed in these plant sources must be interpreted with caution, however, as they can vary significantly from one variety to another, depending on the part of the plant concerned (leaves, flowers, seeds), stress, geographical origin and growing conditions.

Relatively few studies are available on the possible transfer of salicylates ingested via animal feed to animal tissue. In the case of ruminants, although studies have been carried out in the context of veterinary drug registration, no studies have investigated a transfer from animal feed. It is important to note that in polygastrics such as dairy cows, salicylic acid is retained by ion trapping, mainly in the rumen, so transfer to plasma is very limited. This particularity of ruminants means that absorption is reduced by a factor of 1,000, thus lowering concentrations in tissues and milk.

On the basis of a daily ration that respects good agronomic practices, it has been estimated that an adult bovine could consume around 2 g of salicylic acid per day through its feed. Given the information available, these quantities should not lead to MRLs being exceeded.

When ingested in high doses (> 700 mg/litre plasma), salicylates can cause severe toxicity to human health. However, the concentrations found in plants and animal foodstuffs do not represent a risk to human or animal health.

In the opinion of the Scientific Committee, given the current state of knowledge, the presence of salicylic acid residues in milk following the consumption of plants containing high levels of this substance is unlikely, even when plant materials very rich in salicylic acid are consumed.

Salicylates hydrolyse *in vivo* to salicylic acid. As a result, it is impossible to determine whether the salicylic acid detected in a sample was originally salicylic acid, acetylsalicylic acid, methylsalicylic acid or another salicylate. For the same reason, it is currently impossible to distinguish in ruminants between salicylic acid from animal feed (natural) or from a non-feed source (drug treatment or biocide residues). For the Scientific Committee, however, it makes sense to start by investigating sources other than animal feed, given the concentrations that can be found in certain biocides (0.1 to 0.5%) or medicines (660 mg/g of methyl salicylate), compared with those found in animal feed.

7. Recommendations

To the authorities

In cases of non-conformities and in addition to investigations into the improper use of drugs or biocides, the Scientific Committee recommends also checking whether an alfalfa-rich feed was given to the animals and whether willow-based feed supplements were used. In addition, due to the limited number of studies found on the salicylic acid content in alfalfa and due to the importance of alfalfa in animal feed, it may be recommended to conduct further studies on the salicylic acid content of this plant source.

In addition, the use of biocides and compliance with their conditions of use should also be monitored. Ideally, warnings about the use of salicylic acid should be included in the technical data sheets or authorisation documents of biocidal products, pointing out the possibility of it being found in milk or meat.

In order to establish the possible transfer rates from feed to animal products, further studies should be carried out, especially in ruminants.

Furthermore, the Scientific Committee recommends the development of analytical tools allowing to distinguish between a feed (natural) origin and an exogenous origin (medical treatment or contamination with biocides) of salicylic acid residues in animal tissues and more specifically in milk.

In view of the recent detections, the Scientific Committee recommends that trends in the detection of salicylic acid in bovine milk in the coming years be analysed and that epidemiological studies be carried out as thoroughly as possible into the potential causes of MRL exceedance.

For the Scientific Committee,
The Chair,

Dr. Lieve Herman (signed)
31/01/2023

References

American College of Medical Toxicology (2015). Authors unspecified. Guidance document: management priorities in salicylate toxicity. *J Med Toxicol.* 11(1), 149-52. https://doi.org/10.1007/s13181-013-0362-3

Aspirin (2012). LiverTox: Clinical and Research Information on Drug-Induced Liver Injury [Internet]. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases. https://www.ncbi.nlm.nih.gov/books/n/livertox/Aspirin/https://www.ncbi.nlm.nih.gov/books/n/livertox/Aspirin/

Baert, K., Croubels, S., Maes, A., Hillaert, U. Van Calenbergh, S., & De Backer, P. (2004). Comparative metabolic excretion profile of sodium salicylate in broiler chickens and homing pigeons. *J. vet. Pharmacol. Therap.* 27, 123-127. https://doi.org/10.1111/j.1365-2885.2004.00554.x

Beaumier, P. M., Fenwick, J. D., Stevenson, A. J., Weber, M. P., & Young, L. M. (1987). Presence of salicylic acid in Standardbred horse urine and plasma after various feed and drug administrations. *Equine Veterinary Journal* 19(3), 207-213. https://doi.org/10.1111/j.2042-3306.1987.tb01381.x

Bongers, I. E. A., Cramer Bornemann, D., Berendsen, B. J. A., Gerritsen, H. W., van de Schans, M. G. M., Vonsovic, S., & Zuidema, T. (2022). Poster P23: Risk of salicylic acid formation in milk during sample storage. Poster presented at: Euro Residue IX; May 22-25, 2022; St. Michielsgestel, the Netherlands.

Booth, N. H., & McDonald, L. E. (1991). Veterinary Pharmacology and Therapeutics (6th edition). Iowa State University Press / AMES.

Casteels-Van Daele, M., Van Geet, C., Wouters, C., & Eggermont, E. (2000). Reye syndrome revisited: a descriptive term covering a group of heterogeneous disorders. *Eur J Pediatr.* 159(9), 641-648. https://doi.org/10.1007/pl00008399

Ciejka, M., Nguyen, K., Bluth, M. H., & Dubey, E. (2016). Drug toxicities of common analgesic medications in the emergency department. *Clinics in Laboratory Medicine* 36(4), 761-776. https://doi.org/10.1016/j.cll.2016.07.003

Coetzee, J. F., Gehring, R., Bettenhausen, A. C., Lubbers, B. V., Toerber, S. E., Thomson, D. U., Kukanich, B., & Apley, M. D. (2007). Attenuation of acute plasma cortisol response in calves following intravenous sodium salicylate administration prior to castration. *J. vet. Pharmacol. Therap.* 30, 305-313. https://doi.org/10.1111/j.1365-2885.2007.00869.x

Durnas, C., & Cusack, B. J. (1992). Salicylate intoxication in the elderly. Recognition and recommendations on how to prevent it. *Drugs Aging.* 2(1), 20-34. Https://doi.org/10.2165/00002512-199202010-00004

El-Katony, T. M., El-Bastawisy, Z. M., & El-Ghareeb, S. S. (2019). Timing of salicylic acid application affects the response of maize (*Zea mays* L.) hybrids to salinity stress. *Heliyon* 5, e01547. https://doi.org/10.1016/j.heliyon.2019.e01547

Environment and Climate Change Canada, Health Canada (2020). Draft screening assessment – Salicylates group. Chemical Abstracts Service Registry Numbers 69-72-7; 87-22-9; 118-56-9; 68917-75-9; 84012-15-7. https://www.canada.ca/en/environment-climate-change/services/evaluating-existing-substances/draft-screening-assessment-salicylates-group.html

European Agency for the Evaluation of Medicinal Products (1999). Summary report EMEA/MRL/695/99-FINAL of November 1999: Acetylsalicylic acid, sodium acetylsalicylate, acetylsalicylic acid dl-lysine and carbasalate calcium.

European Medicines Agency (2004). Summary report EMEA/MRL/897/04-FINAL of February 2004: Sodium salicylate (extension to oral use in bovine and porcine).

European Medicines Agency (2013). European public MRL assessment report EMA/CVMP/71291/2012 of 15 January 2013. Sodium salicylate (turkeys).

European Medicines Agency (2016). European public MRL assessment report EMA/CVMP/454104/2016 of 12 December 2016. Aluminium salicylate, basic (bovine, caprine, Equidae and rabbit) – after provisional maximum residue limits (MRLs).

Fragnière, C., Serrano, M., Abou-Mansour, E., Métraux, J.-P., & L'Haridon, F. (2011). Salicylic acid and its location in response to biotic and abiotic stress. *FEBS Letters* 585, 1847-1852. https://doi.org/10.1016/j.febslet.2011.04.039

Fecka, I. (2009). Qualitative and quantitative determination of hydrolysable tannins and other polyphenols in herbal products from meadowsweet and dog rose. *Phytochemical Analysis* 20, 177-190. https://doi.org/10.1002/pca.1113

Fitzpatrick, S. R., Garvey, M., Flynn, J., O'Brien, B., & Gleeson, D. (2021). The effect of disinfectant ingredients on teat skin bacteria associated with mastitis in Irish dairy herds. Irish Veterinary Journal 74(1), 1-12. https://doi.org/10.1186/s13620-020-00179-7

Harbourne, N., Marete, E., Jacquier, J. C., & O'Riordan, D. (2009). Effect of drying methods on the phenolic constituents of meadowsweet (*Filipendula ulmaria*) and willow (*Salix alba*). *LWT – Food Science and Technology* 42, 1468-1473. https://doi.org/10.1016/j.lwt.2009.05.005

Hardman, J. G., Limbird, P. B., & Bilman, A. G. (2006). Goodman and Gilman's The Pharmacological Basis of Therapeutics. 11th ed. New York, NY: McGraw-Hill, p. 688.

Iqbal, Y., Ponnampalam, E. N., Suleria, H. A. R., Cottrell, J. J., & Dunshea, F. R. (2021). LC-ESI/QTOF-MS profiling of chicory and lucerne polyphenols and their antioxidant activities. *Antioxidants* 10, 932. https://doi.org/10.3390/antiox10060932

Keszycka, P. K., Szkop, M., & Gajewska, D. (2017). Overall content of salicylic acid and salicylates in food available on the European market. *Journal of Agricultural and Food Chemistry* 65, 11085-11091. https://doi.org/10.1021/acs.jafc.7b04313

Krummen, M., Hilkert, A. W., Juchelka, D., Duhr, A., Schlüter, H.-J., & Pesch, R. (2004). A new concept for isotope ratio monitoring liquid chromatography/mass spectrometry. *Rapid Communications in Mass Spectrometry* 18, 2260-2266. https://doi.org/10.1002/rcm.1620

Lavie, C. J., Howden, C. W., Scheiman, J., & Tursi, J. (2017). Upper Gastrointestinal Toxicity Associated With Long-Term Aspirin Therapy: Consequences and Prevention. *Current Problems in Cardiology* 42(5), 146-164.

Petrek, J., Havel, L., Petrlova, J., Adam, V., Potestil, D., Babula, P., & Kizek, R. (2007). Analysis of salicylic acid in willow barks and branches by an electrochemical method. *Russian Journal of Plant Physiology* 54(4), 553-558. https://doi.org/10.1134/S1021443707040188

Pociecha, E., Plazek, A., Janowiak, F., Waligorski, P., & Zwierzykowski, Z. (2009). Changes in abscisic acid, salicylic acid and phenylpropanoid concentrations during cold acclimation of androgenic forms of Festulolium (*Festuca pratensis* × *Lolium multiflorum*) in relation to resistance to pink snow mould (*Microdochium nivale*). *Plant Breeding* 128, 397-403. https://doi.org/10.1111/j.1439-0523.2009.01664.x

Pozniak, B., Switala, M., Jaworski, K., Okoniewski, P., & Niewinski, P. (2013). Comparative pharmacokinetics of acetylsalicylic acid and sodium salicylate in chickens and turkeys. *British Poultry Science* 54(4), 538-544. https://doi.org/10.1080/00071668.2013.809403

Protasiuk, E., & Olejnik, M. (2018). Determination of salicylic acid in feed using LC-MS/MS (2018). *J Vet Res* 62, 303-307. https://doi.org/10.2478/jvetres-2018-0044

Protasiuk, E., & Olejnik, M. (2020). Residues of salicylic acid and its metabolites in hen plasma, tissues and eggs as a result of animal treatment and consumption of naturally occurring salicylates. *Food Additives & Contaminants: Part A*, https://doi.org/10.1080/19440049.2020.1744740

PubChem CID 2244; Authors unspecified. Aspirin. https://pubchem.ncbi.nlm.nih.gov/compound/2244

Purcarea, C., & Cachita-Cosma, D. (2010). Studies regarding the effects of salicylic acid on maize (*Zea Mays* L.) seedlings under salt stress. *Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii* 20(1), 63-68.

Raskin, I., Skubatz, H., Tang, W., & Meeuse, B. J. D. (1990). Salicylic acid levels in thermogenic and non-thermogenic plants. *Annals of Botany* 66, 369-373. https://doi.org/10.1093/oxfordjournals.aob.a088037

Robertson, G. L., & Kermode, W. J. (1981). Salicylic acid in fresh and canned fruit and vegetables. *J. Sci. Food. Agric.* 32, 833-836. https://doi.org/10.1002/jsfa.2740320813

Scotter, M. J., Roberts, D. P. T., Wilson, L. A., Howard, F. A. C., Davis, J., & Mansell, N. (2007). Free salicylic acid and acetyl salicylic acid content of foods using gas chromatography-mass spectrometry. *Food Chemistry* 105, 273-279. https://doi.org/10.1016/j.foodchem.2007.03.007

Shemi, R., Wang, R., Gheith, E.-S. M. S., Hussain, H. A., Hussain, S., Irfan, M., Cholidah, L., Zhang, K., Zhang, S., & Wang, L. (2021). Effects of salicylic acid, zinc and glycine betaine on morpho-physiological growth and yield of maize under drought stress. *Scientific Reports* 11, 3195. https://doi.org/10.1038/s41598-021-82264-7

Silverman, P., Seskar, M., Kanter, D., Schweizer, P., Métraux J.-P., & Raskin, I. (1995). Salicylic acid in rice. Biosynthesis, conjugation and possible role. *Plant Physiology* 108, 633-639. https://doi.org/10.1104/pp.108.2.633

Vale, A. (2016). Salicylates. *Medicine* 44(3), 199-200. https://doi.org/10.1016/j.mpmed.2015.12.013

Webb, W. K., & Hansen, W. H. (1963). Chronic and subacute toxicology and pathology of methyl salicylate in dogs, rats, and rabbits. *Toxicology and Applied Pharmacology* 5, 576-587. https://doi.org/10.1016/0041-008x(63)90003-6

Zheng, W., Yoo, K. H., Abd El-Aty, A. M., Park, D. H., Choi, J. M., Kim, S. K., Kang, Y. S., Zhang, H. Hacimüftüoglu, A., Bekhit, A. E. D, Wang, J., Shim, J.-H., & Shin, H.-C. (2019). Quantitative

determination of carbasalate calcium derived metabolites, acetylsalicylic acid and salicylic acid, in six animal foods using liquid-liquid extraction method coupled with liquid chromatography-tandem mass spectrometry. *Food Chemistry* 278, 744-750. https://doi.org/10.1016/j.foodchem.2018.11.118

Presentation of the Scientific Committee established at the FASFC

The Scientific Committee is an advisory body established at the Belgian Federal Agency for the Safety of the Food Chain (FASFC) that provides independent scientific opinions on risk assessment and risk management in the food chain, at the request of the Chief Executive Officer of the FASFC, the Minister competent for food safety or at its own initiative. The Scientific Committee is administratively and scientifically supported by the Staff direction for Risk Assessment of the Agency.

The Scientific Committee consists of 22 members who are appointed by Royal Decree on the basis of their scientific expertise in areas related to the safety of the food chain. When preparing an opinion, the Scientific Committee may call upon external experts who are not a member of the Scientific Committee. Similar to the members of the Scientific Committee, they must be able to work independently and impartially. To ensure the independence of the opinions, potential conflicts of interest are managed transparently.

The opinions are based on a scientific assessment of the question. They express the view of the Scientific Committee, which is taken in consensus on the basis of a risk assessment and the existing knowledge on the subject.

The opinions of the Scientific Committee may contain recommendations for food chain control policy or for the stakeholders. The follow up of these **recommendations** for policy is the responsibility of the risk managers.

Questions related to an opinion can be directed to the secretariat of the Scientific Committee: Secretariaat.SciCom@favv-afsca.be.

Members of the Scientific Committee

The Scientific Committee is composed of the following members:

A. Clinquart[1], P. Delahaut, B. De Meulenaer, N. De Regge, J. Dewulf, L. De Zutter, A. Geeraerd Ameryckx, N. Gillard, L. Herman, K. Houf, N. Korsak, L. Maes, M. Mori, A. Rajkovic, N. Roosens, C. Saegerman, M.-L. Scippo, P. Spanoghe, K. Van Hoorde, Y. Vandenplas, F. Verheggen, P. Veys[2], S. Vlaeminck

Conflicts of interest

No conflicts of interest were identified.

[1] Member until December 2021
[2] Member as of January 2022

Acknowledgements

The Scientific Committee thanks the Staff direction for Risk Assessment and the members of the working group for the preparation of the draft opinion, the two deep readers Y. Vandenplas and F. Verheggen, and Pharm. Sofie Rutjens (UGent) for her contribution.

Composition of the working group

The working group was composed of the following members:

Members of the Scientific Committee:	N. Gillard (reporter), P. Delahaut, M.-L. Scippo
External experts:	E. Daeseleire (ILVO), S. Croubels (Ghent University), B. Urbain (FAMHP)
File Manager:	K. Houben

The activities of the working group were monitored by the following members of the administration (as observers): A. E. Popa and C. Rettigner from the Federal Agency for the Safety of the Food Chain.

Legal framework

Act of 4 February 2000, on the creation of the Federal Agency for the Safety of the Food Chain, in particular Article 8;

The Royal Decree of 19 May 2000, on the composition and operating procedures of the Scientific Committee established at the Federal Agency for the Safety of the Food Chain;

The Internal Rules as mentioned in Article 3 of the Royal Decree of 19 May 2000, on the composition and operating procedures of the Scientific Committee established at the Federal Agency for the Safety of the Food Chain, approved by the Minister on 24 September 2020.

Disclaimer

The Scientific Committee at all times reserves the right to modify the opinion, should new information and data become available after the publication of this version of the opinion.