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SCIENCE MEDICINES HEALTH

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Question and answer document on the Guideline on assessment and control of DNA reactive (mutagenic) impurities in veterinary medicinal products (EMA/CVMP/SWP/377245/2016)

Background

The implementation of the Guideline on assessment and control of DNA reactive (mutagenic) impurities in veterinary medicinal products (Ref.1) in 2020 has given rise to requests for clarification relating to its interpretation and application.

This Question and Answer (Q&A) document is intended to provide additional clarification and to promote convergence and improve harmonization of the considerations for assessment and control of DNA reactive (mutagenic) impurities and of the information that should be provided during drug development, marketing authorization applications and/or Master Files.

The scope of this Q&A document follows that of guideline EMA/CVMP/SWP/377245/2016.

“Applicant” is used throughout the Q&A document and should be interpreted broadly to refer to the marketing authorization holder, the filing applicant, the drug product manufacturer, and/or the drug substance manufacturer.

Question 1:

Which impurities fall under the scope of this guideline?

Answer:

Actual and potential impurities that are likely to arise during the synthesis and storage of a new drug substance, and during manufacturing and storage of a new drug product should be assessed.

Potential impurities in the drug substance can include starting materials, reagents, by-products and intermediates in the route of synthesis, from the starting material to the drug substance. Potential degradation products in the drug substance and VMP are those that may be reasonably expected to form during long term storage conditions (including those identified during accelerated stability studies) whose structure is known.



Question 2:

Are the terms “mutagenic potential” and “genotoxic potential” considered interchangeable?

Answer:

No. The terms “mutagenic potential” and “genotoxic potential” are not interchangeable. Mutagenic potential in the context of this guideline refers to the ability of a compound to induce point mutations detectable in a bacterial reverse mutation assay (Ames test) (Ref.2). The terms “genotoxicity” or genotoxic potential, include mutagenicity (which refers to permanent changes in the structure or amount of the genetic material of an organism that can lead to heritable changes in its function; these changes include gene mutations as well as structural and numerical chromosomal alterations), but also include initial DNA damage (as DNA strand breaks or DNA adducts...), which may be reversed by DNA repair processes or other known cellular processes or result in cell death and may not result in permanent alterations in the structure or information content of the surviving cell or its progeny (OECD, 2017a) (Ref.3). This guideline refers only to mutagenic impurities, which are positive in the bacterial mutagenicity test.

Question 3:

How can the TTC-based acceptable intake be converted to a specific concentration limit for an impurity in a veterinary drug substance for food producing animals when the posology of the VMP is expressed as amount of active substance administered per animal (and not per kg bw)?

Answer:

The concentration limit of an impurity in the active substance is calculated based on the TTC for DNA reactive (mutagenic) impurities (0.025 µg/kg bw/day for target animal species), on the body weight of the animal and on the maximum daily dose of the active substance as follows:

$$\text{Impurity limit in active substance (ppm)} = \frac{0.025 \mu\text{g/Kg bw/day} \times \text{Target animal bw (Kg)}}{\text{Daily dose active substance (g/day)}}$$

If the dose of the active substance is expressed as amount of active substance administered per animal (e.g., cloprostenol) standard body weights as in the table below should be used. The concentration limit of the impurity in the active substance should be estimated considering the target species body weight and the highest dose of active substance administered (worst-case). It should be noted that these standard body weights are not intended for use when the amount of active substance is administered on a per kg bw basis.

Table 1 Default body weight values for food producing animals

Animal type	Body weight (Kg) ¹
Cattle	450
Calves	140
Pigs	65
Horses	400

¹Body weights are based upon those detailed in the 'Guideline on environmental impact assessment for veterinary medicinal products in support of the VICH guidelines GL6 and GL38' (EMA/CVMP/ERA/418282/2005-Rev.1- Corr.)

Animal type	Body weight (Kg) ¹
Ponies	250
Sheep	80
Goats	60
Poultry	1.7
Turkey	6.5
Rabbit	1.4

Question 4:

Is this guideline applicable to VMPs intended to be administered to bees? How can the TTC-based limit be estimated in this case? These kinds of products are administered directly to the hive and do not state a dosage per kg body weight.

Answer:

Yes, veterinary medicinal products administered to bees are covered by the scope of the guideline. However, it is acknowledged that in the absence of a recommended dose based on bodyweight, that a pragmatic approach to determining the risk to bees and the consumer is required.

Honeybee colonies are often described as superorganisms which might be defined as any aggregate of individual organisms that behaves like a unified organism (i.e., considered as an organized society that functions as an organic whole).

Therefore, the maximum acceptable intake of mutagenic impurities for target species, 0.025 µg/kg bw, would be considered for the colony as a whole 'superorganism'.

A worst-case default value for colony weight of 15 kg at the time of the treatment is recommended. Other colony weight values should be appropriately justified.

The concentration limit of an impurity in the active substance intended for bees should be estimated as follows:

$$\text{Impurity limit in active substance (ppm)} = \frac{0.025 \mu\text{g/Kg bw/day} \times 15 \text{ Kg}}{\text{Dose active substance (g/day)}}$$

For consumers the "dilution" of the impurity in food-producing target animals is generally assumed to be sufficient so that consumer exposure will normally be below the substance specific acceptable intake (i.e., 0.0025 µg/kg bw/day).

Question 5:

User risk assessment with regard to DNA reactive (mutagenic) impurities. Could the frequency of exposure be taken into account?

Answer:

Yes, but it should be properly justified and assessed on a case-by-case basis bearing in mind that the user is not getting any benefit from the exposure and taking into account the frequency of potential exposure and other sources of exposure, if relevant. When considering the risk to the user, the applicant

needs to ensure that the user's exposure is below the 'virtually safe dose'. That is, a level of human exposure to a genotoxic carcinogen associated with a tumour incidence of ≤ 1 in 10^6 .

Question 6:

What number of expected years of lifetime is considered acceptable for each companion animal species when calculating acceptable intakes in relation to less-than-lifetime (LTL) exposure.

Answer:

A default expected lifetime of 10 years for large dogs (over 40 kg), 15 years for medium dogs (10-39 kg) and 20 years for small dogs (below 10 kg) and 18 years for cats can be used when estimating the risk based on the less-than-lifetime approach. For other companion animals, appropriate justification for the expected lifetime should be provided.

In any case, the applicant should justify the use of the less than lifetime approach and consider factors such as breed-specific mean lifespan, duration of treatment, number of treatment days, severity of indication, limited therapeutic alternatives, etc.

Question 7:

The guideline states: "A higher dose of the impurity applied to the target animal (as described for companion animals) may be justified in exceptional cases. The applicant needs to ensure that consumer exposure is below the 'virtually safe dose' and that consumer safety is not affected. Any deviation from this guidance should be supported with suitable data". Could a higher concentration limit for a mutagenic impurity in a veterinary active substance intended for food-producing animals be refined based on consumer exposure (i.e. 0.5 kg of red meat)?"

Answer:

The guideline states: '*For food-producing animals the TTC-based acceptable intake of 0.025 µg/kg bw/day (or the substance-specific acceptable intake) should not be exceeded, since consumers exposed to residues via food of animal origin are not expected to receive a health benefit. [...]*'

It also notes that '*Estimation of risk based on the LTL approach is not accepted for substances administered to food-producing animals as consideration needs to be given to potential consumer exposure to residues, which could be chronic (potential lifetime exposure is assumed) even if target animal exposure is for only a short duration.*'

Therefore, refinement of the acceptable intake should be an exceptional situation with robust justification.

The TTC limit of a DNA reactive (mutagenic) impurity indicates the acceptable limit of a mutagenic impurity in a drug substance corresponding to an intake with a theoretical 1 in 100,000 excess lifetime cancer risk for target animals (similarly to human patients) and a theoretical 1 in 1,000,000 excess lifetime cancer risk for consumers as well as for users.

As indicated in the guideline, if the amount of the impurity to which the target animal is exposed is below 0.025 µg/kg/day, it can be pragmatically assumed that the amount of impurity ingested by the consumer will be lower than the 'virtually safe dose'. Therefore, the ppm limit for a mutagenic impurity should be estimated as indicated in Appendix 1 (although the example cites "companion animals" the model is also valid for food-producing animals).

The quality guidelines provide advice on how to reduce the level of impurities to acceptable limits.

Only 'In cases where control efforts cannot reduce the level of the mutagenic impurity to below the acceptable limit, a higher limit may be justified based on a benefit/risk analysis'. (See EMA/CVMP/SWP/377245/2016-Table 1)

The benefit/risk analysis should be based on scientific rationale and level of concern, including the drug class effects, the clinical experience (e.g., animal species, drug class effects, clinical considerations), or data on pharmacokinetics of the impurity (e.g., short half-life, poor bioavailability). In these cases, a higher intake of the impurity by the target animal might be justified. When a higher limit is scientifically justified for a mutagenic impurity for a food-producing target species, the acceptable intake (AI) for target animals might be estimated based on consumer exposure via the products derived thereof, i.e. whether consumers are exposed via meat and milk (e.g. cattle) or via meat only (e.g. pigs).

This approach is only valid for administration routes resulting in systemic exposure to the VMP (e.g. oral, i.v.). For products resulting in high local residues (e.g. i.m. or s.c.), the safety of the consumer would not be assured.

Question 8:

Could the frequency of administration in a food-producing target species, for example, a single dose, be acceptable as a risk mitigation measure for the consumer and justify a higher dose of the impurity applied to the food-producing animal (as described for companion animals)?

Answer:

No, the frequency of administration (single dose) in a food-producing target species is not acceptable as a risk mitigation measure for the consumer since the exposure of the consumer is expected to be chronic (lifetime) and depends only on the dosage.

Question 9:

Could it be possible to estimate the impurity limit based on the acceptable daily intake (ADI)?

Answer:

No, the ADI is the daily acceptable intake of active substance used to estimate consumer safety and it may have been established on toxicological endpoints other than mutagenicity/carcinogenicity. It is not related to the amount of mutagenic impurities in the active substance.

Question 10:

Should injection sites be considered in the risk assessment of DNA reactive (mutagenic) impurities; if so, could less-than lifetime considerations be used in relation to injection sites?

Answer:

The less-than-lifetime approach can only be used for companion animals, since consumer exposure is considered chronic. In the risk assessment of DNA reactive (mutagenic) impurities, no difference is made between the injection site and the other edible tissues.

Question 11:

Assessment of multiple impurities. The guideline states "The TTC-based acceptable intakes should be applied to each individual impurity. Higher values of the total mutagenic impurities need to be justified." Could single impurities go significantly higher than the TTC – as long as $n \times$ TTC based acceptable intake is not exceeded and less of another impurity is present in the VMP?

Answer:

No, each individual impurity should be below the respective TTC-based acceptable intake. In case of multiple impurities controlled at the TTC level, the total acceptable intake would be estimated as ' $n \times$ TTC', where ' n ' is the total number of mutagenic impurities.

Question 12:

Should exposure to DNA reactive (mutagenic) impurities generally be controlled at the end of the withdrawal period or "at any time"?

Answer:

DNA reactive impurities should be controlled in the drug substance and in the VMP to guarantee that, the level of these impurities is below the acceptable limit at the batch release and during the entire product lifecycle.

This guideline refers to the control of impurities in drug substances and VMPs. The withdrawal period is based on the depletion of the marker residue and does not take into account the impurities.

Question 13:

In a case where an impurity is demonstrated to be negative in a bacterial mutagenicity assay but positive in a clastogenicity study (e.g., chromosomal aberration test), how would the impurity be classified?

Answer:

In the context of this guideline, if an impurity is negative in a bacterial mutagenicity assay (Ames test), it is considered a Class 5 impurity.

The reporting and control of impurities not falling under the scope of the guideline EMA/CVMP/SWP/377245/2016 should follow VICH GL10/11/18.

If there is information about positive results in a clastogenicity study a more precise evaluation of the genotoxic potential of the impurity should be carried out.

Question 14:

Should non-mutagenic, carcinogenic impurities be controlled according to this guideline?

Answer:

No. Carcinogens that are negative in the bacterial reverse mutation assay are not in the scope of this guideline.

In the context of this guideline, non-mutagenic (negative in the bacterial mutagenicity assay) carcinogenic impurities should be controlled in accordance with the relevant 'impurities' guideline (VICH GL10, GL11 and GL18) (Ref.4, Ref.5, Ref.6), that is, based on the permitted daily exposure.

Question 15:

Should mutagenic, non-carcinogenic impurities be controlled according to this guideline?

Answer:

No. Mutagens that are demonstrated to be non-carcinogenic in appropriate and well-conducted animal bioassays will be treated similarly to Class 5 impurities.

Question 16:

When an out of domain or non-coverage result is obtained from one of the two (Q)SAR models, can the impurity be classified as a Class 5 impurity?

Answer:

No. Out of domain or non-coverage is not considered equivalent to class 5. Additional assessment is warranted.

Given that the relationship between chemical structure and DNA reactivity is well understood, it is unlikely that a structure with mutagenic potential would be associated with an out of domain result. However, expert review can provide reassurance in assignment of such impurities to class 5.

Expert review may include one or a combination of the following (Amberg et. al.,2019) (Ref.7):

1. Comparison to structurally similar analogues for which bacterial reverse mutation assay data are available (read-across approach)
2. Expert review of the chemical structure to determine if there is potential for the chemical to react with DNA.
3. (Q)SAR output from an additional validated model of the same methodology (i.e., expert rule-based or statistical) that generates a prediction that is within its applicability domain.

Question 17:

When the (Q)SAR models indicate the absence of DNA-reactive groups (alerting structure), could the impurity be straightforward classified as Class 5, that is, treat as a non-mutagenic impurity?

Answer:

Yes. A (Q)SAR study performed according to two complementary models resulting in unambiguous results about the absence of DNA-reactive group is sufficient to classify an impurity as Class 5, provided that the evaluation of the impact of Parent/Metabolite structural differences to the mutagenic potential of the substances has been considered (EFSA, 2019) (Ref.8) since the structural changes resulting from metabolic or degradation processes may cause changes in the bacterial mutagenicity test.

Question 18:

If the results of one out of the two (Q)SAR analyses, performed according to two different methodologies, are indeterminate or if the structure of the impurity is out-of-domain, which data should be provided?

Answer:

Additional supporting analysis via Read-across expert review or complementary (Q)SAR assessment of DNA-reactive groups that may arise via metabolic activation is warranted.

For out-of-domain or indeterminate (Q)SAR results, additional supporting analysis to confirm that the impurity lacks any DNA-reactive potential should be also used. This includes a visual assessment of the compound to assure the lack of valid DNA-reactive alerts with plausible mechanisms, taking into consideration any unique alerts from proprietary information or knowledge of metabolic activation (Amberg et al., 2019) (Ref.7)

Question 19:

If an Ames positive impurity is subsequently tested in an appropriate *in vivo* assay and the results are clearly negative, is that sufficient to demonstrate lack of *in vivo* relevance?

Answer:

Yes. A well conducted and scientifically justified *in vivo* study, is sufficient to demonstrate lack of *in vivo* mutagenic relevance. If the results of the *in vivo* study are clearly negative the impurity can be assigned to Class 5.

References

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5. VICH GL11: Impurities in new veterinary medicinal products (EMA/CVMP/VICH/838/99-Rev.1)
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8. Evaluation of the applicability of existing (Q)SAR models for predicting the genotoxicity of pesticides and similarity analysis related with genotoxicity of pesticides for facilitating of grouping and read across. EFSA 2019. doi:10.2903/sp.efsa.2019.EN-1598)