ECHA
EUROPEAN CHEMICALS AGENCY
BOARD OF APPEAL

DECISION OF THE BOARD OF APPEAL OF THE EUROPEAN CHEMICALS AGENCY

9 April 2024

(Substance evaluation – Mutagenicity – Error of assessment – Principle of proportionality – Principle of the protection of legitimate expectations – Article 25 – Duty to state reasons)

Case number	A-008-2022
Language of the case	English
Appellant	Dragon Chemical Europe GmbH, Germany Represented by Ruxandra Cana, Eléonore Mullier and Hannah Widemann Steptoe LLP, Belgium
Interveners	(I) The National Institute of Health, Italy (II) PETA Science Consortium International e.V., Germany
Contested Decision	Decision of 24 May 2022 on the substance evaluation of 5-amino-o-cresol adopted by the European Chemicals Agency pursuant to Article 46 of the REACH Regulation The Contested Decision was notified to the Appellant under annotation number SEV-D-2114596741-38-01/F

THE BOARD OF APPEAL

composed of Antoine Buchet (Chairman), Nikolaos Georgiadis (Technically Qualified Member) and Marijke Schurmans (Legally Qualified Member and Rapporteur)

Registrar: Alen Močilnikar

gives the following

Decision

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1. Background to the dispute

1. In 2020, the Agency included 5-amino-o-cresol[1] (the '**Substance**') in the Community rolling action plan. This decision was based on the opinion of the Member State Committee agreed due to a concern for mutagenicity, sensitisation (skin) and other hazard-based concerns. The Competent Authority of Italy was appointed as the evaluating Member State Competent Authority ('**eMSCA'**).

2. On 18 March 2021, the eMSCA submitted to the Agency a draft decision on substance evaluation in accordance with Articles 46(1) and 52(1) of the REACH Regulation[2].

3. On 6 April 2021, in accordance with Article 50(1), the Agency notified the draft decision to the registrants of the Substance, including the Appellant. The draft decision required those registrants to provide information on a combined *in vivo* mammalian erythrocyte micronucleus test in bone marrow (OECD test guideline ('**TG'**) 474/B.12 EU) and *in vivo* mammalian alkaline comet assay test (OECD TG 489) in liver, gastro-intestinal tract (glandular stomach and duodenum) and urinary bladder performed in rats via the oral route using the Substance.

4. On 12 May 2021, the Appellant submitted comments on the draft decision in accordance with Article 50(1). In its comments, which included an expert statement, the Appellant stated *inter alia* that '*based on a weight of evidence analysis [the Substance] is not considered to be an in vivo genotoxin. […] Based on the totality of the available information, further repeat experimentation is scientifically unnecessary*.'

5. On 3 March 2022, the eMSCA notified a revised draft decision to the competent authorities of the other Member States and to the Agency in accordance with Article 52(1). In the revised draft decision, the requirement to provide information on a micronucleus test (OECD TG 474/B.12 EU) had been removed. According to the revised draft decision, '*it is unlikely that a new in vivo [micronucleus] study would produce different results*.'

6. On 24 May 2022, as no proposals for amendment were submitted, the Agency adopted the Contested Decision in accordance with Article 51(3).

2. Contested Decision

7. The Contested Decision requires the Appellant to submit, by 29 August 2023, information on an *in vivo* mammalian alkaline comet assay test (OECD TG 489) in liver, gastro-intestinal tract (glandular stomach and duodenum) and urinary bladder performed in rats via the oral route using the Substance.

8. The Contested Decision refers to a number of *in vitro* mutagenicity studies, including bacterial reverse mutation tests (OECD TG 471), a gene mutation test on mammalian cells (OECD TG 476), a chromosome aberration test (OECD TG 473) and a micronucleus assay on human lymphocytes (OECD TG 487). The Contested Decision also refers to two '*non-guideline compliant in vitro tests […] reported in the IUCLID*' – a comet assay and a cell transformation assay on Syrian hamster cells.

9. In addition, the Contested Decision states that the following *in vivo* studies relevant to the request for information were available during the substance evaluation process:

   - a mammalian micronucleus test in mice bone marrow cells, performed according to OECD TG 474 (the '**2002 micronucleus test**'),[3]

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[1] EC No 220-618-6; CAS No 2835-95-2.

[2] Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (OJ L 396, 30.12.2006, p. 1). All references to Articles and Annexes hereinafter concern the REACH Regulation unless stated otherwise.

[3] [CONFIDENTIAL].

- a mammalian micronucleus test in mice bone marrow cells, performed according to OECD TG 474 (the '**2005 micronucleus test**'),[4]
- a comet assay on male rats with the analysis of liver, stomach and urinary bladder consisting of two runs (the '**2005 comet assay**'),[5]
- an unscheduled DNA Synthesis ('**UDS**') assay in rat liver according to the OECD TG 486,[6] and
- studies performed under the US National Toxicology Program ('**NTP**'), reported in 2015 (the '**NTP studies**'),[7] including two micronucleus tests and absorption, distribution, metabolism and elimination ('**ADME**') studies.

10. In response to comments from the Appellant on the draft decision, the Contested Decision also refers to an opinion of the Scientific Committee on Consumer Products (the '**SCCP Opinion**').[8]

11. According to the Contested Decision:

'*The available information suggests that the Substance may have a mutagenic effect. However, the available information you reported is not sufficient to clarify the identified concern.*

In particular, the available in vitro data showed the ability for the Substance to induce prevalently clastogenicity and the potential to induce gene mutation and aneugenicity cannot be excluded. The available in vivo data are considered inconclusive.

Therefore a concern on potential mutagenicity of the Substance cannot be excluded […].'

3. Procedure before the Board of Appeal

12. On 23 August 2022, the Appellant filed this appeal.

13. On 25 October 2022, the Agency submitted its Defence.

14. On 8 November 2022, the eMSCA was granted leave to intervene in support of the Agency, and PETA Science Consortium International e.V. ('**PSCI**') was granted leave to intervene in support of the Appellant.

15. On 9 January 2023, the Appellant submitted its observations on the Defence.

16. On 20 January 2023, the eMSCA and PSCI submitted their respective statements in intervention.

17. On 14 February 2023, the Agency submitted its observations on the eMSCA's statement in intervention.

18. On 17 February 2023, the Agency submitted its observations on PSCI's statement in intervention and on the Appellant's observations on the Defence.

19. On 27 February 2023, the Appellant submitted its observations on the eMSCA's statement in intervention.

20. On 28 February 2023, the Appellant submitted its observations on PSCI's statement in intervention.

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4 [CONFIDENTIAL].

5 [CONFIDENTIAL].

6 [CONFIDENTIAL].

7 National Toxicology Program, *NTP Technical Report on the Toxicity Studies of 5-amino-ocresol (cas. N.2835-95-2) administered dermally to f344/NTac rats and B6C3F1/N mice*, 2015.

8 European Commission, *Opinion on 4-Amino-2-hydroxytoluene COLIPA No A27*, SCCP/1001/06, 10 October 2006.

21. On 30 March 2023, the Agency and the Appellant provided the documents requested by the Board of Appeal.

22. On 3 October 2023, a hearing was held as the Board of Appeal considered it to be necessary in accordance with Article 13(1) of the Rules of Procedure[9]. The hearing was held at the Agency's premises. At the hearing, the Appellant, the Agency, and the Interveners made oral submissions and responded to the questions from the Board of Appeal.

4. Form of order sought

23. The Appellant, supported by PSCI, requests the Board of Appeal to:

- annul the Contested Decision,
- order the refund of the appeal fee, and
- take such other or further measures as justice may require.

24. The Agency, supported by the eMSCA, requests the Board of Appeal to dismiss the appeal as unfounded.

5. Assessment of the case

25. The Appellant raises the following pleas in law, alleging that the Agency:

- breached the principle of proportionality, made errors of assessment, and failed to take all relevant information into account in concluding that:
  (a) there is a potential risk related to mutagenicity,
  (b) there is a need to clarify the alleged potential risk, and
  (c) the information requested has a realistic possibility of leading to improved risk management measures;
- breached the Appellant's legitimate expectations;
- breached Article 25; and
- breached the duty to state reasons in the Contested Decision.

5.1. Admissibility of certain pleas raised by PSCI

26. In its observations on PSCI's statement in intervention, the Agency argues that three of the pleas raised by PSCI in its statement in intervention should be dismissed as inadmissible. Those pleas concern:

- the relationship between the REACH Regulation and the Cosmetics Regulation,[10]
- the reliability of the *in vivo* comet assay requested in the Contested Decision, and
- the prior testing of liver toxicity before performing the *in vivo* comet assay as a less onerous measure in the present case.

27. Under the third subparagraph of Article 8(6)(b) of the Rules of Procedure, a statement in intervention must contain the pleas in law and arguments of fact and law relied on.

28. Under Article 8(3) of the Rules of Procedure, an intervention must be limited to supporting or opposing, in whole or in part, the remedy sought by one of the parties.

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[9] Commission Regulation (EC) No 771/2008 laying down the rules of organisation and procedure of the Board of Appeal of the European Chemicals Agency (OJ L 206, 2.8.2008, p. 5).

[10] Regulation (EC) No 1223/2009 of the European Parliament and of the Council on cosmetic products (OJ L 342, 22.12.2009, p. 59).

29. An intervener has the right to set out arguments and pleas independently, in so far as they support the form of order sought by one of the main parties and are not entirely unconnected with the issues underlying the dispute, as defined by the main parties, as that would otherwise change the subject-matter of the dispute.[11]

30. Therefore, when determining the admissibility of the pleas put forward by an intervener, the Board of Appeal must determine whether they are connected with the subject-matter of the dispute, as defined by the main parties.

5.1.1. Plea concerning the relationship between the REACH Regulation and the Cosmetics Regulation

Arguments of the Parties and PSCI

31. In its statement in intervention, PSCI raises a plea related to the relationship between the Cosmetics Regulation and the REACH Regulation. PSCI argues that this furthers the Appellant's pleas.

32. PSCI argues that there was nothing in the available appeal documents, or publicly available information to suggest that the Substance is used in anything other than cosmetics. PSCI also argues that, according to settled case-law, it is able to enter pleas independently.

33. The Agency argues that the Appellant does not challenge the Contested Decision on the basis that the Substance is registered for cosmetic purposes only. According to the Agency, the Intervener's argument is therefore inadmissible.

34. The Appellant states that it did not raise any pleas or arguments related to the relationship between the requirements under the REACH Regulation and the Cosmetics Regulation. The Appellant argues, however, that it did state in its submissions that the Substance is only used as an ingredient in cosmetic products.

Findings of the Board of Appeal

35. In the present case, PSCI's plea related to the relationship between the Cosmetics Regulation and the REACH Regulation was not raised by one of the Parties. Furthermore, that plea is entirely unconnected with the subject-matter of the dispute. Consequently, that plea must be declared inadmissible.

5.1.2. Plea concerning the reliability of the *in vivo* comet assay requested in the Contested Decision

Arguments of the Parties and PSCI

36. In its statement in intervention, PSCI presents arguments related to the reliability of the *in vivo* comet assay requested in the Contested Decision.

37. According to PSCI, the results of a comet assay, such as the one requested in the Contested Decision, are difficult to interpret. It argues that this is directly related to the Appellant's pleas and arguments.

38. The Agency argues that the Appellant does not challenge the reliability of the comet assay in the present proceedings. Consequently, according to the Agency, PSCI's plea is inadmissible.

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[11] See, for example, judgment of 15 June 2005, *Regione autonoma della Sardegna*, T-171/02, EU:T:2005:219, paragraphs 152 and 153; and decision of the Board of Appeal of 30 May 2017, *Manufacture Française des Pneumatiques Michelin*, A-022-2015, paragraph 60; see also paragraph 88 of the Practice directions to parties to appeal proceedings before the Board of Appeal of the European Chemicals Agency (14 March 2023).

39. The Appellant states that it did not directly raise a plea related to the reliability of the comet assay. The Appellant argues, however, that it did present arguments in its appeal related to the challenges of interpreting the results of a comet assay.

Findings of the Board of Appeal

40. PSCI's arguments on the reliability of the comet assay are directly related to the Appellant's arguments concerning the difficulties in interpreting the results of a comet assay. Therefore, PSCI's arguments must be considered as supporting the form of order sought by the Appellant and as being connected with the subject-matter of the dispute.

41. The Agency's plea that PSCI's arguments are inadmissible must therefore be rejected.

5.1.3. Plea concerning prior testing of liver toxicity before performing the *in vivo* comet assay

Arguments of the Parties and PSCI

42. In its statement in intervention, PSCI argues that, in selecting the least onerous measure in the present case, the possible hepatotoxicity of the Substance should have been clarified before requiring a new *in vivo* comet assay. According to PSCI, the repetition of an *in vivo* comet assay, which has already been conducted on the Substance, might, especially in the presence of hepatotoxicity, again yield inconsistent results.

43. PSCI argues that its argument supports the Appellant's plea that the comet assay requested in the Contested Decision is not the least onerous measure.

44. The Agency argues that the possibility of performing tiered testing was not raised by the Appellant. Therefore, according to the Agency, PSCI's arguments on tiered testing must be dismissed as inadmissible.

45. The Appellant argues that PSCI's arguments concern the issue of the least onerous course of action in the present case. According to the Appellant, PSCI arguments are therefore directly connected to the Appellant's pleas.

Findings of the Board of Appeal

46. The Appellant does not specifically argue that the Agency should have requested the Appellant to examine further the possible hepatotoxicity of the Substance before requiring information on an *in vivo* comet assay. However, the Appellant does raise the argument that in the available comet assay on the Substance potential hepatotoxicity could be a masking effect in the liver and therefore genotoxicity in the liver could not be completely ruled out.

47. Furthermore, under its plea alleging that the Agency breached Article 25, the Appellant argues that the Agency did not have recourse to the least onerous measure to address the concern identified.

48. PSCI's arguments related to the tiered approach to testing must therefore be considered as supporting the form of order sought by the Appellant and as being connected with the subject-matter of the dispute.

49. The Agency's plea that PSCI's arguments are inadmissible must therefore be rejected.

5.2. Substance of the case

5.2.1. First plea: The Agency breached the principle of proportionality, erred in its assessment and failed to take relevant information into account by concluding that there is a potential risk related to mutagenicity

Arguments of the Parties and the Interveners

50. The Appellant, supported by PSCI, argues that it is disproportionate to require the Appellant to repeat an existing vertebrate animal study. According to the Appellant, the available information allows for it to be concluded that there is no concern related to mutagenicity for the Substance.

- *Potential risk*

51. The Appellant argues that the Agency committed errors of assessment and failed to take relevant information into account in concluding that there is a potential risk for the Substance related to mutagenicity. The Appellant does not challenge the potential exposure to the Substance.

52. The Appellant argues that the Agency made errors in its assessment of the available *in vivo* information. According to the Appellant, the Agency also failed to conduct an evaluation of all the available *in vitro* and *in vivo* information, taken together in a weight of evidence approach, using expert judgment.

53. The Appellant argues that the available *in vivo* information on the Substance is sufficient to clarify and exclude any potential concern related to the mutagenic properties of the Substance observed in the *in vitro* studies.

54. The Appellant argues that the Agency erred in finding that the available micronucleus tests show that there is a potential concern related to mutagenicity. According to the Appellant, contrary to the conclusion in the Contested Decision, those tests are valid and demonstrate that the Substance is negative for induction of micronuclei in the bone marrow.

55. The Appellant argues that the Agency also erred in concluding that the available comet assay and UDS assay are inconclusive as regards a potential concern for mutagenicity.

56. The Appellant argues that the conclusion that there is no concern for mutagenicity is supported by the SCCP Opinion, the findings in the NTP studies and the expert statement included in its comments on the draft decision.

57. The Agency, supported by the eMSCA, disputes the Appellant's arguments.

- *Need to clarify a potential risk related to mutagenicity*

58. The Appellant argues that there is no need to investigate a concern related to mutagenicity as there is already sufficient clear, robust and conclusive *in vitro* and *in vivo* information available to conclude on this endpoint.

59. The Appellant argues that evaluation by expert judgment should have been carried out by the eMSCA and the Agency in accordance with the Agency's Guidance.[12] According to the Appellant, if the eMSCA and the Agency had performed such an evaluation, it would have led to the conclusion that the available data confirms the lack of mutagenicity of the Substance *in vivo.*

[12] ECHA, *Guidance on Information Requirements and Chemical Safety Assessment, Chapter R.7a: Endpoint specific guidance*, Version 6.0, July 2017, p. 560.

60. The Appellant argues that if the Agency considered that the individual studies submitted in the registration dossier were not sufficiently conclusive, it should have assessed that information using a weight-of-evidence approach in accordance with Section 1.2. of Annex XI.

61. The Agency, supported by the eMSCA, disputes the Appellant's arguments.

- *Realistic possibility that the requested information will lead to improved risk management measures*

62. The Appellant argues that repeating the *in vivo* comet assay does not have a realistic possibility of leading to improved risk management measures. According to the Appellant, there are valid data available which are sufficient to conclude on the mutagenicity endpoint and therefore on the need to take risk management measures in relation to that endpoint.

63. The Agency, supported by the eMSCA, disputes the Appellant's arguments.

Findings of the Board of Appeal

64. To comply with the principle of proportionality, measures adopted by the Agency must not exceed the limits of what is appropriate and necessary to attain the objectives legitimately pursued by that measure; when there is a choice between several appropriate measures, recourse must be had to the least onerous, and the disadvantages caused must not be disproportionate to the aims pursued.[13]

65. To demonstrate the necessity of a request for information under substance evaluation, the Agency must establish that:

- there are grounds for considering that, based on a combination of information on potential hazard and potential exposure, a substance constitutes a potential risk to human health or the environment,
- the potential risk needs to be clarified, and
- the requested information, needed to clarify the concern, has a realistic possibility of leading to improved risk management measures.[14]

66. To request information under substance evaluation, it is not necessary for the Agency to demonstrate an actual risk, only a potential risk. The aim of requesting additional information under substance evaluation is to clarify the risk.[15]

67. This is consistent with the different types of risk that must be taken into account at different stages of the processes established by the REACH Regulation.

68. This is also consistent with the European Union Courts' interpretation of the precautionary principle according to which a preventive measure may be taken only if the risk, although the reality and extent thereof have not been 'fully' demonstrated by conclusive scientific evidence, appears nevertheless to be adequately backed up by the scientific data available at the time the measure was taken.[16]

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[13] Decision of the Board of Appeal of 10 May 2022, *LANXESS Deutschland*, A-002-2021, paragraph 88.

[14] Judgment of 20 September 2019, *BASF Grenzach* v *ECHA*, T-125/17, EU:T:2019:638, paragraph 276; decision of the Board of Appeal of 17 January 2023, *SCAS Europe*, A-009-2021, paragraph 75.

[15] Judgment of 20 September 2019, *BASF Grenzach* v *ECHA*, T-125/17, EU:T:2019:638, paragraphs 269 to 273; decision of the Board of Appeal of 22 March 2022, *Campine*, A-003-2020, paragraph 110.

[16] Judgment of 11 September 2002*, Pfizer Animal Health* v *Council*, T-13/99, EU:T:2002:209, paragraph 144; decision of the Board of Appeal of 17 December 2019, *BASF and Kemira*, A-003-2018 to A-005-2018, paragraphs 84 to 87.

69. A request for further information under substance evaluation cannot be triggered by a purely hypothetical risk[17] or by a failure to prove the lack of any risk.[18]

70. It is the Agency's responsibility to justify a request for further information under substance evaluation by demonstrating that the three conditions of the necessity test referred to in paragraph 65 above are met.

71. When an appellant challenges such an information request, it must show that the Agency erred in its conclusions on one or more of those three conditions.

72. In assessing the Appellant's pleas that the Agency committed errors of assessment, it must therefore be examined whether the arguments put forward by the Appellant demonstrate that the Agency made errors and failed to take all relevant information into account in concluding that those three conditions are met in the present case.[19]

73. The principle of proportionality also requires that the requested information must be capable of achieving its objective. Therefore, in order to demonstrate the appropriateness of an information request in the context of substance evaluation, the Agency must be able to establish that the potential risk posed by the substance can be clarified by the requested information.[20]

(a) Potential risk

74. In the Contested Decision, the Agency identified a potential risk related to mutagenicity – both chromosomal aberration and gene mutation.

75. According to the Contested Decision, an *in vivo* comet assay was requested as it is capable of, amongst other things, investigating the potential hazard related to both chromosomal aberration and gene mutation.

76. The Appellant does not challenge the Agency's conclusion in the Contested Decision that there is potential exposure to the Substance.

77. However, the Appellant argues that the Agency committed an error in finding that, based on the available information taken as a whole, there is a potential hazard in relation to both chromosomal aberration and gene mutation.

(i) Potential hazard related to chromosomal aberration

78. The Appellant's registration dossier contains information on *in vitro* studies which, on their own, indicate a potential hazard related to chromosomal aberration.[21]

79. Vertebrate animal studies are needed to clarify positive results observed in *in vitro* mutagenicity tests.[22] Furthermore, according to the Agency's Guidance, '*if different findings are obtained in vitro and in vivo, in general, the results of in vivo tests indicate a higher degree of reliability.*'[23]

80. To investigate the findings of the available *in vitro* studies, the Appellant's registration dossier contains the following *in vivo* data relevant to chromosomal aberration performed according to OECD TG 474:

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[17] See, to that effect, judgment of 5 February 2004, *Commission* v *France*, C-24/00, EU:C:2004:70, paragraph 56.

[18] See, to that effect, judgment of 21 October 2003, *Solvay* v *Council,* T-392/02, EU:T:2003:277, paragraph 130.

[19] Decision of the Board of Appeal of 12 January 2021, *Chemours Netherlands*, A-007-2019, paragraph 40.

[20] Decision of the Board of Appeal of 15 January 2019, *3v Sigma,* A-004-2017, paragraph 88.

[21] See paragraph 8 above.

[22] See, for example, ECHA, *Guidance on Information Requirements and Chemical Safety Assessment, Chapter R.7a: Endpoint specific guidance*, Version 6.0, July 2017, p. 565.

[23] ECHA, *Guidance on Information Requirements and Chemical Safety Assessment, Chapter R.7a: Endpoint specific guidance*, Version 6.0, July 2017, p. 562.

- the 2002 micronucleus test, and
- the 2005 micronucleus test.

81. The version of OECD TG 474 adopted on 21 July 1997 was the applicable version of that test guideline at the time the 2002 and 2005 micronucleus tests were performed. The version of that test guideline adopted on 29 July 2016 was the version applicable at the time of the adoption of the Contested Decision.

82. In its comments on the draft decision, the Appellant also referred to the findings of the NTP studies, the SCCP Opinion and an expert statement which it submitted with its comments on the draft decision.

83. According to the Appellant, the *in vivo* studies investigated and excluded the concern identified in the Contested Decision.

84. However, according to the Contested Decision, the available *in vivo* information is inconclusive and therefore cannot exclude the potential mutagenic effects observed in the *in vitro* data.

- *The 2005 micronucleus test*

85. According to the robust study summary of the 2005 micronucleus test, under the test conditions reported, the Substance did not induce micronuclei in bone marrow polychromatic erythrocytes of the mouse. In other words, the test was negative.

86. Paragraph 49 of the 2016 version of OECD TG 474 states that '*there is no requirement for verification of a clear positive or clear negative response*'.

87. The Agency does not contest the validity of the 2005 micronucleus test. However, according to the Contested Decision, the results of the 2005 micronucleus test are inconclusive because of:

- insufficient exposure of the Substance to bone marrow, and
- lack of investigation of effects at the first site of contact.

88. Each of these points will be examined separately below.

Bone marrow exposure

89. According to the Contested Decision, '*[s]ome signs of systemic toxicity, such as reduction of spontaneous activity, ruffled fur and orange colored urine, was observed in the animals treated at 250 and 500 mg/kg bw. The ratio of PCE/NCE in [bone marrow] was not decreased after treatment with the Substance as compared to the control. A negative result in a [micronucleus test] in the absence of clear toxicity to target organ (toxicity to Bone marrow cell, PCE/NCE ratio) should be considered inconclusive*.'

90. Consequently, according to the Contested Decision, one of the reasons why the 2005 micronucleus test should be considered inconclusive is that there was insufficient exposure to the bone marrow in that test.

91. However, for the following reasons, the Agency committed an error in concluding that the results of the 2005 micronucleus test are inconclusive because of insufficient bone marrow exposure.

92. First, no threshold for bone marrow exposure was set in the 1997 version of OECD TG 474. According to paragraph 22 of that version of the test guideline, '*[t]he highest dose may also be defined as a dose that produces some indication of toxicity of the bone marrow (e.g. a reduction in the proportion of immature erythrocytes among total erythrocytes in the bone marrow or peripheral blood).*'

93. Similarly, according to paragraph 48 of the 2016 version of OECD TG 474, there is no threshold for bone marrow exposure, and various ways of demonstrating proof of bone marrow exposure are accepted. Paragraph 48 of the 2016 version of OECD TG 474 states:

'*Providing that all acceptability criteria are fulfilled, a test chemical is considered clearly negative if, in all experimental conditions examined:*

(a) None of the treatment groups exhibits a statistically significant increase in the frequency of micronucleated immature erythrocytes compared with the concurrent negative control,

(b) There is no dose-related increase at any sampling time when evaluated by an appropriate trend test,

(c) All results are inside the distribution of the historical negative control data (e.g. Poisson-based 95% control limits), and

(d) Bone marrow exposure to the test substance(s) occurred.

[…] Evidence of exposure of the bone marrow to a test substance may include a depression of the immature to mature erythrocyte ratio or measurement of the plasma or blood levels of the test substance. In case of intravenous administration, evidence of exposure is not needed. Alternatively, ADME data, obtained in an independent study using the same route and same species can be used to demonstrate bone marrow exposure. Negative results indicate that, under the test conditions, the test chemical does not produce micronuclei in the immature erythrocytes of the test species.'

94. Second, the Contested Decision acknowledges that the available data show that there was systemic exposure, and therefore bone marrow exposure, to the Substance in the 2005 micronucleus test.

95. According to the Contested Decision, '*[i]n your comments to the draft decision, you claimed that the systemic availability of the Substance after oral application based on toxicokinetic and NTP studies is demonstrated. The eMSCA considers that while <u>some systemic exposure is demonstrated</u>, no conclusion can be reached regarding a possible effect at the first site of contact where the exposure is assumed to be higher*' (emphasis added).

96. The eMSCA also acknowledged during the present proceedings that the 2005 micronucleus test was performed at the maximum tolerated dose ('**MTD**') of 500 mg/kg bw, and that this dose produced signs of systemic toxicity. In other words, the eMSCA also acknowledges that there was bone marrow exposure in that study.

97. The Agency also acknowledged during the present proceedings that the Substance was systemically available in the organism and therefore perhaps also to the target organ (bone marrow) in the available micronucleus tests without showing evident mutagenicity. In fact, the Agency acknowledged in its written submissions in the present case that the micronucleus tests submitted by the Appellant demonstrate that the Substance did not induce genotoxic effects specifically in the bone marrow.

98. Third, the Appellant submitted ADME studies[24] which, as acknowledged in the Contested Decision, demonstrated the systemic availability of the Substance after oral administration relevant to the micronucleus tests.

[24] Paragraph 9 above.

First site of contact

99. According to the Contested Decision, '*[N]o conclusion can be drawn on the effects at first site of contact (i.e. GI tract after oral administration) where the Substance concentration could be higher than at the distal site. Then, the eMSCA considers that it cannot […] conclude whether the Substance administered at a higher dose could be genotoxic at the site of first contact.*'

100. In other words, the Agency considers that the potential risk related to chromosomal aberration raised by the *in vitro* data has not been clarified by the 2002 and 2005 micronucleus tests because, apart from the lack of decrease on the ratio of PCE/NCE in bone marrow, the first site of contact remains unexamined. Where the Substance is administered orally, as in the 2005 micronucleus test, the first site of contact are the upper parts of the digestive system.

101. According to paragraph 1 of the 1997 version of OECD TG 474, '*the mammalian in vivo micronucleus test is used for the detection of damage induced by the test substance to the chromosomes or the mitotic apparatus of erythroblasts by analysis of erythrocytes as sampled in bone marrow and/or peripheral blood cells of animals, usually rodents*' (emphasis added).

102. Similarly, according to paragraph 3 of the 2016 version of OECD TG 474, '*[t]he mammalian in vivo micronucleus test is used for the detection of damage induced by the test chemical to the chromosomes or the mitotic apparatus of erythroblasts. The test evaluates micronucleus formation in erythrocytes sampled either in the bone marrow or peripheral blood cells of animals, usually rodents*' (emphasis added).

103. Neither the 1997 nor the 2016 version of OECD TG 474 refer to a requirement to examine the first site of contact.

104. Therefore, the 2005 micronucleus study was performed according to OECD TG 474 as there was an examination of micronucleus formation in erythrocytes sampled in the bone marrow. Consequently, the Agency committed an error in concluding that the results of the 2005 micronucleus test are inconclusive because that test did not examine the first site of contact.

105. The Agency did not therefore demonstrate that the conclusion in the robust study summary for the 2005 micronucleus test that that test was negative is incorrect. In general, there is no requirement to verify a clear positive or negative response in such a micronucleus test.[25] However, under substance evaluation, the Agency may be able to demonstrate a concern, based on all the available evidence, which requires further testing. For example, where a substance reaches the tissue under investigation, and if the test is negative, it may still be necessary to consider other relevant tissues, for example the first site of contact.

106. The Agency argues that it would be necessary to examine mutagenic effect at the first site of contact – the upper parts of the digestive system – because the exposure is higher at that point than the systemic exposure in the organism. However, this argument could apply to all *in vivo* mutagenic testing conducted by oral administration and does not explain why there is a concern at the first site of contact which is specific to the Substance and which would make it necessary in the present case to deviate from the normal course of action set out in paragraphs 101 to 104 above. This is particularly the case in view of the negative results observed in the 2005 micronucleus test, in which systemic exposure to the Substance was demonstrated.

[25] See paragraph 49 of the 2016 version of OECD TG 474.

107. Consequently, the Agency did not demonstrate why it would be necessary to investigate effects at the first site of contact despite the negative findings in the 2005 micronucleus test. The Agency did not demonstrate why there is a concern related to chromosomal aberration at the first site of contact which is specific to the Substance and which requires further investigation under substance evaluation.

- *The 2002 micronucleus test*

108. According to the robust study summary of the 2002 micronucleus test, under the test conditions reported, the study did not induce micronuclei in bone marrow polychromatic erythrocytes of the mouse. In other words, the study was negative.

109. The robust study summary also states that the dose selection was based on a dose range-finding assay in which a dose of 200 mg/kg bw, administered by intraperitoneal injection, was observed to induce signs of toxicity.

110. According to the Contested Decision, in relation to the 2002 micronucleus test, '*although the systemic availability of the Substance can be assumed because of the application route used [intraperitoneal route], no conclusion can be reached, due the very low dosage used […]*'.

111. The Appellant argues that the results of the 2002 micronucleus test should not be disregarded on the ground that '*a very low dosage*' was used in that study because:

- the intraperitoneal route exposes the liver to a higher concentration than the oral route, and
- the 2005 micronucleus test was performed at the MTD and higher doses led to animal deaths.

112. According to paragraph 24 of the 1997 version of OECD TG 474, '[*t]he test substance is usually administered by gavage using a stomach tube or a suitable intubation cannula, or by intraperitoneal injection*'.

113. However, according to paragraph 35 of the 2016 version of OECD TG 474, '*intraperitoneal injection is generally not recommended since it is not an intended route of human exposure, and should only be used with specific scientific justification.*'

114. In the present case, the Appellant did not set out such a scientific justification in its registration dossier for using intraperitoneal injection.

115. Since the mode of administration used in the 2002 micronucleus test was through intraperitoneal injection, the results of that test were not on their own sufficient to clarify the potential risk related to chromosomal aberration. However, the results of that test do not contradict or call into question the negative results of the 2005 micronucleus test. Consequently, the results of the 2002 micronucleus test cannot affect the conclusion drawn from the 2005 micronucleus test that the Substance does not constitute a potential risk related chromosomal aberration.

- *Conclusion on the potential risk related to chromosomal aberration*

116. In view of paragraphs 85 to 107 above, the Agency committed an error in concluding that the results of the 2005 micronucleus test were inconclusive, rather than clearly negative as reported in the robust study summary. Specifically, the Agency committed an error in concluding that the results of the 2005 micronucleus test are inconclusive due to insufficient bone marrow exposure.

117. Furthermore, in the absence of other results to the contrary, a negative result in a well-performed micronucleus test is sufficient to exclude a concern related to chromosomal aberration.

118. In addition, the Agency did not demonstrate that it is necessary to investigate a potential hazard related to chromosomal aberration at the first site of contact despite the negative results of the 2005 micronucleus test.[26]

119. Therefore, the Agency breached the principle of proportionality by failing to demonstrate that the requested information is necessary in order to investigate a potential risk related to chromosomal aberration.

120. The Contested Decision must therefore be annulled in so far as it concludes that there is a concern related to chromosomal aberration. As a result, it is not necessary to examine the Appellant's other pleas in so far as they relate to this part of the Contested Decision. For the same reasons, it is also not necessary to examine the Appellant's arguments related to the SCCP Opinion and the NTP studies in so far as they concern a potential risk related chromosomal aberration.

121. However, as stated in paragraph 75 above, the comet assay was requested in the Contested Decision in order to examine a potential risk related to both chromosomal aberration and gene mutation. In this respect, it is not disputed that the 2002 and 2005 micronucleus tests are not appropriate to investigate a potential risk related to gene mutation. Consequently, it is necessary to examine the Appellant's claims that the Agency committed errors in finding that there is a potential risk related to gene mutation.

(ii) Potential hazard related to gene mutation

122. The Appellant's registration dossier contains information on *in vitro* studies[27] which, on their own, indicate a potential hazard related to gene mutation.

123. As stated in paragraph 79 above, vertebrate animal studies are needed to clarify positive results observed in *in vitro* mutagenicity tests.[28] Furthermore, according to the Agency's Guidance, '*if different findings are obtained in vitro and in vivo, in general, the results of in vivo tests indicate a higher degree of reliability.*'[29]

124. The Appellant's registration dossier contains information on the following *in vivo* studies which are relevant to a potential hazard related to gene mutation:

- the 2005 comet assay, and
- the UDS assay.

125. According to the Appellant, the available *in vivo* data allow to conclude that there is no potential hazard related to gene mutation.

126. To support its claim that there is no potential hazard related to gene mutation, the Appellant also refers to the SCCP Opinion and an expert statement which it submitted with its comments on the draft decision.

127. According to the Agency, the available information is inconclusive and there remains a potential hazard related to gene mutation.

- *Weight-of-evidence approach*

128. The Appellant argues that the available information should not be examined in isolation and should be considered together in a weight-of-evidence approach in accordance with Section 1.2. of Annex XI.

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[26] See paragraphs 106 and 107 above.

[27] See paragraph 8 above.

[28] See, for example, ECHA, *Guidance on Information Requirements and Chemical Safety Assessment, Chapter R.7a: Endpoint specific guidance*, Version 6.0, July 2017, p. 565.

[29] ECHA, *Guidance on Information Requirements and Chemical Safety Assessment, Chapter R.7a: Endpoint specific guidance*, Version 6.0, July 2017, p. 562.

129. However, Annex XI applies to the standard information requirements in the Annexes and the dossier evaluation procedure. Contrary to the Appellant's arguments, in the substance evaluation procedure, the Agency is not required to assess weight-of-evidence adaptations against the criteria set out in Section 1.2. of Annex XI.[30]

130. Nonetheless, in exercising its discretion, the Agency is required to take into consideration all the relevant factors and circumstances of the situation the act is intended to regulate. In this respect, Article 47(1) requires that '*an evaluation of a substance shall be based on all relevant information submitted on that particular substance and on any previous evaluation under [Title VI – Evaluation]*.'[31]

131. Consequently, in examining the Appellant's plea, it is necessary to consider whether the Agency took into account all the available evidence before deciding, based on that evidence as a whole, that there is a concern related to mutagenicity which requires further investigation.[32]

- *The 2005 comet assay*

132. The 2005 comet assay included in the Appellant's registration dossier was conducted prior to the adoption of OECD TG 489. According to the Contested Decision, the authors of the study '*declared they followed the robust method, scientifically agreed, reported in* [CONFIDENTIAL]'[33]. That report also serves as a reference in the adopted version of OECD TG 489.

133. The Agency does not contest that the results of the 2005 comet assay may be relevant to the assessment of the hazard related to gene mutation.[34]

134. However, in the Contested Decision and during the present proceedings, the Agency highlighted deficiencies in the conduct of the 2005 comet assay. Because of those deficiencies, the Agency considered that the study was not capable of clarifying the potential hazard related to gene mutation. The main deficiency identified by the Agency concerned the fact that the four dose levels (including the control group) applied in the first run of that study were not used in the second run.

135. During the present proceedings, the Agency also noted deficiencies in the historical control data used in the 2005 comet assay. The Agency also identified several differences between the study performed by the Appellant and a study performed according to the current version of OECD TG 489, including the number of cells to be analysed in the study.

Dose levels applied in the 2005 comet assay

136. In the first run of the 2005 comet assay, the doses applied were of 0, 500, 1 000 and 2 000 mg/kg bw respectively. A parallel histopathology study was also conducted to clarify whether the test substance caused cytotoxic effects in the tissues investigated.

137. In the first run, in the liver, a statistically significant increase in mean tail length, mean tail moment, and mean tail intensity at low-dose was observed, as well as an increase of the mean of tail moment at mid-dose, compared to the concurrent vehicle control. In additon, a hepatotoxic response was observed at the high dose (2 000 mg/kg bw) while no cytotoxicity was reported at any other dose. The author of the study attributed this finding to a loss of cells due to hepatotoxicity. In the stomach

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30 Decision of the Board of Appeal of 22 March 2022, *Campine*, A-003-2020, paragraph 118.

31 Decision of the Board of Appeal of 13 December 2017, *Akzo Nobel Chemicals*, A-023-2015, paragraph 152.

32 Decision of the Board of Appeal of 30 June 2017, *Evonik Degussa and Others*, A-015-2015, paragraph 123.

33 [CONFIDENTIAL].

34 See, on that point, decision of the Board of Appeal of 6 June 2018, *SI Group and Others*, A-006-2016, paragraph 174.

and the urinary bladder the mean tail moment showed similar results at the low-dose. No dose-response was observed in any of the treated organs, in all parameters analysed.

138. In the robust study summary of the 2005 comet assay, the results of the first run of that study were summarised as follows:

'*After administration of 0, 500, 1000 and 2000 mg/kg in the first part of the study, inconclusive results were obtained. Mean tail length, mean tail moment and mean tail intensity in liver and stomach cells were increased inversely to dose*.'

139. Since the results of the first run were inconclusive, the comet assay was run again by applying doses of 0 and 2 000 mg/kg bw. In the robust study summary, the results of the second run were summarised as follows:

'*10 male rats each were treated at 0 and 2000 mg/kg bw and cells of the liver, stomach and urinary bladder epithelium investigated in the Comet Assay. No biologically relevant and statistically significant increases of tail length, tail moment and tail intensity were determined after treatment with 2000 mg/kg in cells of any of the investigated tissues.*'

140. Therefore, the Substance was considered not to be mutagenic in the comet assay *in vivo* to stomach and urinary bladder. It was also reported that '*primary genotoxicity of test substance to rat liver could not be excluded*.'

141. The Appellant argues that following the results of the comet assay the liver was the only target organ for which further clarification was required. According to the Appellant, the remaining concern for the liver was clarified through the negative results of the UDS assay.

142. It is not disputed that, generally, there is no requirement to verify a clearly positive or negative response in a correctly conducted *in vivo* comet assay.

143. It is also not disputed that the 2005 comet assay did not produce a clearly positive response.

144. However, for the following reasons, the results of the 2005 comet assay are not clearly negative in the stomach, urinary bladder and liver.

145. In the first run of the 2005 comet assay, the results were reported as inconclusive. In the second run, only the high dose was tested. This is despite the fact that statistically significant increases were observed in the low and mid-doses in the first run. Consequently, the effects observed in the first run were not fully clarified in the second run.

146. The Agency therefore did not commit an error in concluding in the Contested Decision that the results of the 2005 comet assay are inconclusive. The 2005 comet assay did not allow a conclusion to be reached on the concern related to gene mutation in the stomach, urinary bladder, or the liver. The comet assay requested in the Contested Decision, if performed using the appropriate doses, may clarify the inverse dose response and the effects observed at the low and mid-dose in the first run of the 2005 comet assay.

147. Consequently, the results of the 2005 comet assay were not capable of clarifying the potential risk related to gene mutation.

148. This finding is not called into question by the Appellant's arguments that its conclusion that no additional information is required on gene mutation was confirmed in the SCCP Opinion, by the results of the UDS assay, and by the expert statement submitted with the comments on the draft decision.

- *The UDS assay*

149. For the following reasons, contrary to the Appellant's arguments, the negative results of the UDS assay are not sufficient to clarify the potential risk for gene mutation in the liver.

150. According to paragraph 1 of OECD TG 486, '*[t]he purpose of the unscheduled DNA synthesis (UDS) test with mammalian liver cells in vivo is to identify substances that induce DNA repair in liver cells of treated animals*'.

151. According to the Agency's guidance, '*[a] negative result in a UDS assay alone is not a proof that a substance does not induce gene mutation*.'[35]

152. The Agency's guidance states further that '*[t]he UDS test is an indicator test measuring DNA repair of primary damage in liver cells but not a surrogate test for gene mutations per se. The UDS test can detect some substances that induce in vivo gene mutation because this assay is sensitive to some (but not all) DNA repair mechanisms. However not all gene mutagens are positive in the UDS test and it is thus useful only for some classes of substances. A positive result in the UDS assay can indicate exposure of the liver DNA and induction of DNA damage by the substance under investigation but it is not sufficient information to conclude on the induction of gene mutation by the substance.*' [36]

153. Paragraph 6 of the OECD TG 486 also describes the limits of this test method:

'*The detection of a UDS response is dependent on the number of DNA bases excised and replaced at the site of the damage. Therefore, the UDS test is particularly valuable to detect substance-induced "longpatch repair" (20-30 bases). In contrast, "shortpatch repair" (1-3 bases) is detected with much lower sensitivity. Furthermore, mutagenic events may result because of non-repair, misrepair or misreplication of DNA lesions. The extent of the UDS response gives no indication of the fidelity of the repair process. In addition, it is possible that a mutagen reacts with DNA but the DNA damage is not repaired via an excision repair process. The lack of specific information on mutagenic activity provided by the UDS test is compensated for by the potential sensitivity of this endpoint because it is measured in the whole genome.*'

154. Consequently, as stated in the Agency's Guidance referred to above, the information obtained from the UDS assay on its own cannot clarify the gene mutation concern in the liver. Furthermore, that concern was not clarified in the 2005 comet assay.[37] As a result, the Appellant has not demonstrated that the Agency committed an error in finding that the available information, taken together, shows that there is a potential hazard related to gene mutation.

- *The SCCP Opinion*

155. The SCCP Opinion was referred to by the Appellant in its comments on the draft decision.

156. The conclusion on mutagenicity set out in the SCCP Opinion is as follows:

'*4-amino-2-hydroxytoluene was not mutagenic in Salmonella typhimurium. However, it induced mutations in mouse lymphoma L5178Y cells in vitro (small colonies indicating clastogenicity), micronuclei in human lymphocytes in vitro, and DNA strand breaks in Chinese hamster V79 cells, without metabolic activation. In vivo, 4-amino-2-hydroxytoluene did not induce micronuclei in mouse bone marrow, or unscheduled*

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[35] ECHA, *Guidance on Information Requirements and Chemical Safety Assessment, Chapter R.7a: Endpoint specific guidance*, Version 6.0, July 2017, p. 572.

[36] ECHA, *Guidance on Information Requirements and Chemical Safety Assessment, Chapter R.7a: Endpoint specific guidance*, Version 6.0, July 2017, pp. 571 and 572.

[37] See paragraphs 132 to 148 above.

DNA synthesis in rat hepatocytes. In an in vitro comet assay, the test substance was found positive; however, its major metabolite was found negative. However, primary genotoxicity of 4-amino-2-hydroxytoluene could not be excluded in rat liver, where the comet assay indicated an increase in DNA strand breakage.

On the basis of the available data, the substance has no relevant mutagenic potential in vivo.'

157. At the outset, it should be noted that the evidence relevant to mutagenicity examined in the SCCP Opinion was also examined in the Contested Decision.

158. In this respect, as set out above,[38] the Appellant has not demonstrated that the Agency made an error in concluding that the available *in vivo* studies are not capable of clarifying the potential hazard related to gene mutation.

159. In the Contested Decision, the Agency considered that the conclusion in the SCCP Opinion was not justified for the following reasons:

'*[T]he eMSCA notes that also the SCCP in its conclusions states that: "primary genotoxicity of 4-amino-2-hydroxytoluene [the Substance] could not be excluded in rat liver, where the comet assay indicated an increase in DNA strand breakage." The final SCCP conclusion "On the basis of the available data, the substance has no relevant mutagenic potential in vivo" is not explained and appears to the eMSCA not justified.'*

160. It is clear from that part of the Contested Decision that both the eMSCA, when conducting the substance evaluation, and subsequently the Agency, in adopting the Contested Decision, took into account the SCCP Opinion and found there to be a contradiction between the reporting of the results of the available information and the conclusion that *'[o]n the basis of the available data, the substance has no relevant mutagenic potential in vivo*'.

161. It should also be noted that the terms of reference of the SCCP Opinion read as follows:

'*Does the Scientific Committee on Consumer Products (SCCP) consider 4-amino-2-hydroxytoluene safe for consumers, when used in oxidative hair dye formulations with a concentration on the scalp of maximum 1.5% taking into account the scientific data provided?'*

162. It is therefore clear that the SCCP Opinion evaluated the risks connected with a particular consumer use, namely the risks incurred from the exposure of consumers through a particular use of the Substance.

163. Consequently, the finding in the SCCP Opinion that *'the substance has no relevant mutagenic potential in vivo'* (emphasis added) does not mean that the Substance does not present any other concern to human health, for example in relation to workers or other consumer uses.

- *The expert statement submitted with the Appellant's comments on the draft decision*

164. It should be noted that the evidence evaluated in the expert statement submitted with the comments on the draft decision is also examined in the Contested Decision.

165. The fact that the expert statement submitted by the Appellant comes to a different conclusion to that reached by the experts from the Agency and the eMSCA is not, in itself, sufficient to lead to a conclusion that the Agency committed an error of assessment. Rather than demonstrating an error of assessment, the expert statement shows a difference of scientific opinion on the assessment of the available information.

[38] See paragraphs 132 to 154 above.

166. The data available to the Agency in a substance evaluation process may lead to differences of opinion between experts when assessing that data. One of the main purposes of substance evaluation is to clarify potential risks and thereby help resolve the differences of opinion between experts or clarify a potential risk over which there is a consensus.[39]

167. In relation to the main deficiency observed in the conduct of the 2005 comet assay regarding the doses used in the second run of that study,[40] the expert statement does not provide clarifications or justifications as to why the second run of that study was not conducted at the low and mid doses.

(iii) Conclusion on the Appellant's plea that the Agency failed to demonstrate a potential risk related to gene mutation

168. In view of paragraphs 122 to 167 above, the Appellant has not demonstrated that the Agency failed to take into account all the available evidence before deciding, based on that evidence as a whole, that there is a concern related to gene mutation which requires further investigation.

169. Furthermore, the Appellant did not demonstrate that the Agency committed an error in finding in the Contested Decision that, based on the evidence as a whole, there is a potential risk related to gene mutation that requires further investigation. The Appellant did not demonstrate that, even when the available evidence is taken together, the Agency committed an error in finding that the potential risk related to gene mutation in the stomach, urinary bladder and liver had not been clarified through the 2005 comet assay, the UDS assay, the SCCP Opinion and the expert statement submitted with the comments on the draft decision.

170. The failure to conduct the second run in the 2005 comet assay at the low and mid doses meant that the study did not clarify the inconclusive results observed in the first run of that study.[41] The 2005 comet assay was therefore not capable of clarifying the results of the available *in vitro* studies[42] which indicated a potential hazard related to gene mutation. This potential risk was also not clarified by the other information available in the Appellant's registration dossier and submitted during the substance evaluation process.

(b) Need to clarify the potential risk related to gene mutation and whether the requested information has a realistic possibility of leading to improved risk management measures

171. According to Section 1.3. of Appendix A to the Contested Decision, '*the available information is not sufficient to conclude on the potential hazard. Consequently, further data is needed to clarify the potential risk related to the mutagenicity of the Substance*'.

172. In Section 1.4. of Appendix A to the Contested Decision, the Agency sets out the further risk management measures that it considers could result from the requested information. Those measures include classification and labelling and the potentially resulting improved measures at manufacturing sites, better waste management and revised instructions on safe use.

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[39] Decision of the Board of Appeal of 22 March 2022, *Campine*, A-003-2020, paragraph 135.

[40] See paragraphs 134 to 148 above.

[41] See paragraphs 132 to 148 above.

[42] See paragraph 8 above.

173. The Appellant argues that there is no need to clarify a potential risk related to mutagenicity and there is not a realistic possibility that the requested information will lead to improved risk management measures. According to the Appellant, this is because there is sufficient information to conclude that there is no potential risk related to mutagenicity. The Appellant's arguments are therefore based on the premise that there is no potential risk related to mutagenicity. The Appellant does not challenge the possible improved risk management measures set out in the Contested Decision that may result from the requested information.

174. For the reasons set out in paragraphs 122 to 170 above, the Appellant did not demonstrate that the Agency committed an error in finding in the Contested Decision that, based on the available information taken as a whole, the Substance poses a potential risk related to gene mutation that requires further investigation.

175. Consequently, the Appellant's arguments that the Agency committed an error in finding that there is a need to clarify the potential risk and that the requested information has a realistic possibility of leading to improved risk management measures must be rejected.

176. That conclusion is not called into question by the Appellant's argument that the comet assay was a standard information requirement at the time the Contested Decision was adopted. In this respect, it must be noted that the Contested Decision does not address the issue of whether the 2005 comet assay submitted by the Appellant in its registration dossier was sufficient to meet the standard information requirements of the REACH Regulation. Similarly, the Appellant does not argue that the Contested Decision should have been adopted under the compliance check procedure under Article 41 rather than the substance evaluation procedure under Article 46.

177. In any case, under substance evaluation requests for information are based on a potential risk and may go beyond the standard information requirements set out in Annexes VII to X.

(c) Conclusion on the first plea in relation to gene mutation

178. It follows from the reasons set out in paragraphs 122 to 177 above that the Appellant did not demonstrate that the Agency breached the principle of proportionality, erred in its assessment and failed to take relevant information into account in concluding that there is a potential risk related to gene mutation.

179. The Appellant also failed to demonstrate that the Agency committed an error in concluding that there is a need to clarify the potential risk related to gene mutation and that the requested information has a realistic possibility of leading to improved risk management measures.

180. The Appellant's first plea in relation to gene mutation must therefore be rejected.

181. Since the Contested Decision must be annulled in so far as it concerns the potential risk related chromosomal aberration only, it is necessary to examine the remainder of the Appellant's pleas in so far as they are relevant to the potential risk related to gene mutation.

5.2.2. Second plea: Breach of the principle of the protection of legitimate expectations

Arguments of the Parties and the Interveners

182. The Appellant, supported by PSCI, argues that, based on the Agency's guidance documents, it had a legitimate expectation that the Agency would not require a repetition of the comet assay.

183. The Appellant argues that, according to the Agency's Guidance,[43] the comet assay and the UDS assay, which are available in the registration dossier for the Substance, are appropriate to clarify the positive results observed in the *in vitro* gene mutation studies.

184. The Appellant argues that the Contested Decision is not consistent with the applicable test guidelines. This is because it requires the comet assay to be repeated in order to cover different parts of the gastrointestinal tract compared to the existing comet assay – specifically, the glandular stomach and the duodenum rather than the stomach. The Appellant argues that, contrary to the Contested Decision, such an approach is not in line with OECD TG 489.

185. The Appellant argues that scientific literature does not support the Agency's position that mutagenic carcinogens induce tumours in different target organs. According to the Appellant, primary targets have been investigated and it is unlikely that testing additional tissues, which are typically not viewed as target sites, will change the conclusion.

186. The Appellant argues that the Contested Decision is inconsistent with the guidance in that it questions the results in the existing *in vivo* comet assay, whereas under OECD TG 489 results should only be considered positive for mutagenicity if there is a dose-related increase. According to the Appellant, that was not the case for the existing comet assay as no dose dependent tail intensity was observed.

187. The Agency, supported by the eMSCA, disputes the Appellant's arguments.

Findings of the Board of Appeal

188. The right to rely on the principle of the protection of legitimate expectations presupposes that precise, unconditional and consistent assurances originating from authorised, reliable sources have been given to the person concerned by the competent authorities of the European Union. That right applies to any individual in a situation in which a European Union institution, body or agency, by giving that person precise assurances, has led that individual to entertain well-founded expectations. Precise, unconditional and consistent information, in whatever form it is given, constitutes such an assurance.[44]

189. Contrary to the Appellant's arguments, the Contested Decision does not conclude that the results of the 2005 comet assay are positive. The Agency rather concludes in the Contested Decision that, overall, the results of that assay are inconclusive.

190. Furthermore, it is because the results of the 2005 comet assay were inconclusive, and therefore unable to clarify the concern related to mutagenicity observed in the available *in vitro* studies, that the Agency required a new comet assay to be performed. The Agency did not require the Appellant to perform a new comet assay on the grounds that the 2005 comet assay was not performed according to OECD TG 489.

191. In relation to the Appellant's arguments that it had legitimate expectations, based on the Agency's guidance,[45] that it would not be required to provide information on a new comet assay, it should be noted that the Agency's guidance outlines the test guidelines to be followed to meet the standard information requirements set out in the Annexes. In addition, as the Contested Decision was adopted under the substance evaluation procedure rather than the compliance check procedure, the Agency does not take a position in that decision on whether the 2005 comet assay conforms with the applicable version of OECD TG 489.[46]

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[43] ECHA, '*Three recently approved in vivo genotoxicity test guidelines*' (revised in February 2018).

[44] See, for example, decision of the Board of Appeal of 22 March 2022, *Campine*, A-003-2020, paragraph 207.

[45] See paragraph 183 above.

[46] See paragraph 176 above.

192. In addition, and in any event, the Contested Decision does not contradict the statement in that guidance that an adequate comet assay (OECD TG 489) and, if necessary, an adequate UDS assay (OECD TG 486) are appropriate studies to clarify positive results observed in *in vitro* gene mutation studies.

193. Indeed, the Agency requested the comet assay to clarify the positive results observed in the *in vitro* studies included in the Appellant's registration dossier. In this respect, the Agency concluded in the Contested Decision that the 2005 comet assay was not adequate to clarify the concern. This is because of the inconclusive findings of that assay. As a result, it was necessary to request additional information to clarify the concern.

194. The Agency did not contest the conclusion that the results of the UDS study were negative. The Agency rather concluded that the negative result in the UDS assay is not sufficient on its own to clarify the concern related to gene mutation in the liver. In this respect, bearing in mind that the results of the 2005 comet assay were inconclusive, the Appellant did not provide any other evidence capable of supporting a conclusion that there is no potential risk related to gene mutation in the liver.

195. Furthermore, as stated above, the Agency did not commit an error in concluding that the results of the 2005 comet assay are inconclusive.[47] In those circumstances, the Appellant cannot claim to have legitimate expectations based on the Agency's Guidance that it would not be required to perform a new *in vivo* comet assay to clarify the potential risk identified in the *in vitro* data where the results of the comet assay included in its registration dossier were found to be inconclusive and therefore inadequate to clarify the concern related to gene mutation.

196. For the following reasons, the Appellant's arguments related to the requirement in the Contested Decision to perform the comet assay to cover different parts of the gastrointestinal tract than those covered in the 2005 comet assay - specifically, glandular stomach and duodenum, rather than the stomach - must also be rejected.

197. First, the available information is insufficient to clarify the concern related to gene mutation.[48] The Agency therefore did not commit an error in requesting additional information to clarify that concern. It is also not disputed that a comet assay performed according to OECD TG 489 is appropriate to clarify a concern related to gene mutation.

198. Second, in selecting the tissues to be examined in the comet assay the Agency acted in accordance with paragraph 42 of OECD TG 489 in requiring the investigation of the liver, gastro-intestinal tract (glandular stomach and duodenum) and urinary bladder.

199. It not disputed that it is appropriate to investigate the liver and urinary bladder. However, contrary to the Appellant's arguments, and for the reasons given in paragraphs 132 to 154 above, the potential risk related to gene mutation in those organs has not been clarified by the existing *in vivo* data. The Appellant has therefore not demonstrated that it is inappropriate in the present case to perform a new comet assay to investigate effects in the liver and urinary bladder.

200. Consequently, the comet assay requested in the Contested Decision is not required to investigate the potential risk related to gene mutation in the gastrointestinal tract (glandular stomach and duodenum) only.

201. Third, investigation of the glandular stomach and the duodenum is foreseen in OECD TG 489. According to paragraph 42 of that test guideline: '*in some cases examination of a site of direct contact (for example, for orally-administered substances the glandular stomach or duodenum/jejunum, or for inhaled substances the lungs) may be most relevant*'.

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[47] See paragraph 146 above.

[48] See paragraph 170 above.

202. In Section 2.1(b) of Appendix A to the Contested Decision, the Agency provides justification as to why effects in the gastrointestinal tract (glandular stomach and duodenum) should be investigated: '*There are several expected or possible variables between the glandular stomach and the duodenum (different tissue structure and function, different pH conditions, variable physico-chemical properties and fate of the Substance, and probable different local absorption rates of the Substance and its possible breakdown product(s)). In light of these expected or possible variables, it is necessary to analyse both tissues to ensure a sufficient evaluation of the potential for genotoxicity at the site of contact in the gastro-intestinal tract.*'

203. Therefore, the Appellant cannot claim to have legitimate expectations that it would not be required to examine the glandular stomach and duodenum in the requested comet assay.

204. Fourth, the Appellant's argument that a scientific article submitted with its Notice of Appeal does not support the idea that mutagenic carcinogens induce tumours in different target organs cannot demonstrate that the Agency breached the Appellant's legitimate expectations. Such literature from sources external to the Agency cannot constitute precise, unconditional and consistent assurances from the Agency.

205. In view of paragraphs 188 to 204 above, the Appellant's second plea in relation to gene mutation must be rejected.

5.2.3. Third plea: Breach of Article 25

Arguments of the Parties and the Interveners

206. The Appellant, supported by PSCI, argues that under Article 13 of the Treaty on the Functioning of the European Union (**TFEU**), Article 25 of the REACH Regulation, and Directive 2010/63/EU,[49] as few animals as possible should be used in testing.

207. The Appellant argues that the Contested Decision was adopted in breach of the European Union's animal protection objectives and Article 25. According to the Appellant, this is because there is already sufficient information to conclude on the concern related to mutagenicity.

208. According to the Appellant, the Contested Decision requires the Appellant to repeat a comet assay which will only confirm the conclusion that the Substance is not genotoxic *in vivo*.

209. The Appellant argues that requesting the repetition of the comet assay is not the last resort in this case. According to the Appellant, if the Agency had questions concerning the interpretation of the results of the existing *in vivo* studies, there were other options to ensure that additional vertebrate animal testing only took place as a last resort, such as applying a weight-of evidence approach or seeking additional expert input.

210. The Appellant states that, according to the Contested Decision, additional vertebrate animal testing – a germ cell genotoxicity study – may still be required if no clear conclusion can be made on germ cell mutagenicity.

211. PSCI argues that, in selecting the least onerous measure in the present case, the possible hepatotoxicity of the Substance should have been resolved before requiring a new *in vivo* comet assay. According to PSCI, the repetition of an *in vivo* comet assay, which has already been conducted on the Substance, might, especially in the presence of hepatotoxicity, again yield inconsistent results.

212. The Agency, supported by the eMSCA, disputes the Appellant's arguments.

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[49] Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes (OJ L 276, 20.10.2010, p. 33).

Findings of the Board of Appeal

213. Article 13 of the TFEU provides, amongst other things, that in formulating and implementing the European Union's internal market policies, the Union and the Member States must, since animals are sentient beings, pay full regard to the welfare requirements of animals. The REACH Regulation contains a number of provisions which take into account the welfare of animals. This includes Article 25(1) which provides '*[i]n order to avoid animal testing, testing on vertebrate animals for the purposes of [the REACH] Regulation shall be undertaken only as a last resort […]*'.[50]

214. The protection of animal welfare is therefore an important consideration in the framework of European Union legislation and the REACH Regulation in particular. Where the Agency requires additional testing pursuant to a substance evaluation it must ensure that vertebrate animals are used only as a last resort. The Agency's actions should not run counter to the principles of Directive 2010/63/EU.[51]

215. As stated above, vertebrate animal studies are needed to clarify the positive results observed in the *in vitro* mutagenicity tests.[52]

216. The Agency did not commit an error in concluding that, despite the available *in vivo* data, there remains a potential risk related to gene mutation.[53]

217. As a result, the Appellant's argument that no additional *in vivo* testing is required to investigate the concern related to gene mutation must be rejected.

218. It is clear from the wording of the Contested Decision that alternatives to the comet assay – although in relation to concerns for both chromosomal aberration and gene mutation – were considered by the Agency. The Agency concluded that the comet assay was the least onerous measure because there is no equally suitable alternative method, amongst the *in vivo* tests, available to obtain information that would clarify the potential mutagenicity hazard. The Contested Decision also states that '*[t]wo possible alternative in vivo are available, the TGR assay (OECD TG 488) and the spermatogonial assay (OECD TG 483). The TGR is not the most adequate because it is only able to detect gene mutation in vivo and is also a more expensive test. The spermatogonial assay is able to detect clastogenic effects but only on germ cells*.'

219. For the reasons given above, the Appellant's arguments that in the present case a weight-of-evidence approach and the use of expert opinion would have constituted suitable alternatives to animal testing must also be rejected.[54]

220. The Appellant's statement that the Contested Decision clearly states that a further follow-up test on vertebrate animals may be requested after the results of the comet assay are submitted also does not demonstrate that the Agency breached Article 25 by requesting a comet assay in the Contested Decision.

221. PSCI's argument that the Agency could have requested an *in vitro* study to clarify the possible hepatoxicity of the Substance must also be rejected. The *in vitro* testing of hepatocytes suggested by PSCI would only provide information on the concern related to the liver. Consequently, it would still be necessary to clarify the concern related to the other organs identified in the Contested Decision through *in vivo* studies.

222. In view of paragraphs 213 to 221 above, the third plea in relation to gene mutation must be rejected.

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[50] See for example, decision of the Board of Appeal of 22 March 2022, *Campine*, A-003-2020, paragraph 234.

[51] See for example, decision of the Board of Appeal of 22 March 2022, *Campine*, A-003-2020, paragraph 235.

[52] See paragraphs 79 and 123 above.

[53] See paragraph 174 above.

[54] See paragraphs 122 to 181 above.

5.2.4. Fourth plea: Breach of the duty to state reasons

Arguments of the Parties and the Interveners

223. The Appellant, supported by PSCI, argues that the Agency failed to comply with its duty to state reasons in the Contested Decision. The Appellant argues that the Contested Decision does not explain why a repeat of the comet assay is necessary despite the conclusions of the SCCP Opinion.

224. The Appellant argues that the Contested Decision also fails to set out any assessment of the coherence of requesting a repeat of the existing comet assay with the Agency's duties under Article 25 and the European Union's animal welfare objectives.

225. The Agency, supported by the eMSCA, disputes the Appellant's arguments.

Findings of the Board of Appeal

226. Under Article 130, the Agency must state reasons for all decisions it takes under the REACH Regulation. The duty to state reasons is an essential procedural requirement which is enshrined in the second paragraph of Article 296 of the TFEU and is included in Article 41(2)(c) of the Charter of Fundamental Rights of the European Union as part of the right to good administration.[55]

227. A statement of reasons must be appropriate to the act at issue and must disclose in a clear and unequivocal fashion the reasoning followed by the institution, body or agency which adopted the measure in question. This must be done in such a way as to enable the persons concerned to ascertain the reasons for the measure and to enable the Board of Appeal and the European Union judicature to exercise their powers of review.[56] Whether a statement of reasons is adequate or not depends on all the circumstances of a case, in particular, the content of the measure in question, the nature of the reasons given and the interest which the addressees of the measure, or other parties to whom it is of direct and individual concern, may have in obtaining explanations.[57]

228. For the following reasons, the Appellant's arguments that the Agency breached its duty to state reasons in the Contested Decision must be rejected.

229. First, the Contested Decision provides the Appellant with sufficient information to ascertain the reasons why the Agency considers the available information to be insufficient to clarify the potential risk related to gene mutation identified in the available *in vitro* data. In particular, it is clear from the Contested Decision why the Agency considers the results of the 2005 comet assay to be inconclusive, and as such, incapable of clarifying the concern related to gene mutation.

230. Second, for the reasons given above,[58] the Contested Decision contains sufficient reasoning to demonstrate that the SCCP Opinion was taken into account. The reasoning in the Contested Decision is also sufficient to enable the Appellant to ascertain the reasons why the Agency considers that there is a potential risk related to gene mutation despite the conclusions of the SCCP Opinion.

231. Third, as stated above, the Contested Decision clearly contains reasoning regarding the need to perform testing on vertebrate animals and the available alternatives.[59]

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55 Decision of the Board of Appeal of 29 June 2021, *SNF*, A-001-2020, paragraph 134.

56 See, by analogy, judgment of 21 December 2016, *Club Hotel Loutraki and Others* v *Commission*, C-131/15 P, EU:C:2016:989, paragraph 46.

57 See judgment of 10 March 2016, *HeidelbergCement* v *Commission*, C-247/14 P, EU:C:2016:149, paragraph 16.

58 See paragraphs 155 to 163 above.

59 See paragraph 218 above.

232. In view of paragraphs 226 to 231 above, the Appellant's fourth plea in relation to gene mutation must be rejected.

5.2.5. Conclusion on the appeal as regards the concern related to gene mutation

233. As all the Appellant's pleas in relation to the concern related to gene mutation have been rejected, the appeal must be dismissed in so far as it relates to that concern.

5.3. Result

234. The Contested Decision must be annulled in so far as it concludes that there is a potential hazard related to chromosomal aberration. However, the Appellant's pleas on the potential hazard related to gene mutation have been rejected.

235. For the following reasons, the parts of the Contested Decision regarding the concerns related to (i) chromosomal aberration and (ii) gene mutation are clearly severable.

236. First, the comet assay was requested in the Contested Decision to examine a potential risk related to both chromosomal aberration and gene mutation.

237. Second, under the Annexes to the REACH Regulation, there are separate endpoints on mutagenicity, which includes separate information requirements for both chromosomal aberration (for example, Section 8.4.2. of Annex VIII) and gene mutation (for example, Section 8.4.1. of Annex VII and Section 8.4.3. of Annex VIII).

238. Third, if clearly positive results are observed in the comet assay requested in the Contested Decision which reach the necessary level of severity, this can lead, based on the evidence as a whole, to the necessary classification (for example, germ cell mutagen category 2) and resulting improved risk management measures.

239. Under Article 93(3), the Board of Appeal is competent to replace a substance evaluation decision with its own decision or remit the case to the Agency for further action.[60] In the present case, as the Contested Decision is partially erroneous, the Board of Appeal would therefore be competent to replace the Contested Decision with a decision seeking to clarify the potential risk related to gene mutation only.

240. However, before replacing a substance evaluation decision with its own decision, the Board of Appeal must examine whether the available evidence allows it to do so. In addition, when examining whether it can replace an Agency decision, the Board of Appeal must take into account the procedure for adopting Agency decisions under the substance evaluation process set out in Articles 50 to 52, and in particular the role of the various actors in that procedure.[61]

241. It is therefore necessary to examine whether the evidence available to the Board of Appeal is sufficient to replace the Contested Decision with its own decision.

242. In the Contested Decision, the most appropriate study to meet the objectives of the Contested Decision was assessed on the basis of potential hazards related to both chromosomal aberration and gene mutation. The Agency requested the comet assay in the Contested Decision because, amongst other reasons, it considered that the test is the most appropriate to clarify the concerns related to both chromosomal aberration and gene mutation. The Contested Decision also states that '*[t]wo possible alternative in vivo are available, the TGR assay (OECD TG 488) and the spermatogonial assay (OECD TG 483). The TGR is not the most adequate because it is only able to detect gene mutation in vivo and is also a more expensive test. The spermatogonial assay is able to detect clastogenic effects but only on germ cells*'.

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[60] Judgment of 20 September 2019, *BASF Grenzach* v *ECHA*, T-125/17, EU:T:2019:638, paragraph 117.

[61] Judgment of 20 September 2019, *BASF Grenzach* v *ECHA*, T-125/17, EU:T:2019:638, paragraph 118. See also decision of the Board of Appeal of 10 May 2022, *Lanxess and Schirm*, Case A-002-2021, paragraph 109.

243. The Agency and the eMSCA argued during the present proceedings that, if the Board of Appeal were to find that the Agency had not demonstrated a concern related to chromosomal aberration, it would give the Appellant the option of performing either the comet assay or a TGR assay, as both are capable of clarifying a concern related to gene mutation.

244. However, the most appropriate and least onerous test to address the concern related to gene mutation only was not discussed during the decision-making procedure in the present case. Therefore, the relevant actors were not given the opportunity to comment on this issue. Consequently, the Board of Appeal does not possess sufficient information to be able to decide whether the comet assay (OECD TG 489), or another test, is the most appropriate test in the present case.

245. In view of the reasons set out in paragraphs 234 to 244 above, considering the procedure for adopting Agency decisions under the substance evaluation process set out in Articles 50 to 52, and in particular the role of the various actors in that procedure, the case must be remitted to the Agency for further action.

6. Refund of the appeal fee

246. In accordance with Article 10(4) of Commission Regulation (EC) No 340/2008 on the fees and charges payable to the European Chemicals Agency pursuant to the REACH Regulation,[62] the appeal fee must be refunded if the appeal is decided in favour of an appellant. As the Contested Decision has been annulled, the appeal fee must be refunded.

On those grounds,

THE BOARD OF APPEAL

hereby:

1. **Annuls the Contested Decision.**
2. **Remits the case to the competent body of the Agency for further action.**
3. **Decides that the appeal fee is refunded.**

Antoine BUCHET
Chairman of the Board of Appeal

Alen MOČILNIKAR
Registrar of the Board of Appeal

[62] OJ L 107, 17.4.2008, p. 6.