~~Confidential~~

Helsinki, 14 April 2023

Addressee
Registrant of 13752-51-7 Registration as listed in Appendix 3 of this decision

Date of submission of the dossier subject to this decision
02/06/2022

Registered substance subject to this decision ("the Substance")
Substance name: 4-[(morpholinothio)thioxomethyl]morpholine
EC/List number: 237-335-9

Decision number: Please refer to the REACH-IT message which delivered this communication (in format CCH-D-XXXXXXXXXX-XX-XX/F)

DECISION ON A COMPLIANCE CHECK

Under Article 41 of Regulation (EC) No 1907/2006 (REACH), you must submit the information listed below by ***21 October 2025***.

Requested information must be generated using the Substance unless otherwise specified.

Information required from all the Registrants subject to Annex VII of REACH

1. In vivo mammalian alkaline comet assay combined with in vivo mammalian erythrocyte micronucleus test (triggered by Annex VII, Section 8.4., column 2) also requested below.

Information required from all the Registrants subject to Annex VIII of REACH

2. In vivo mammalian alkaline comet assay combined with in vivo mammalian erythrocyte micronucleus test (triggered by Annex VIII, Section 8.4., column 2) in rats, or if justified, in mice, oral route. For the comet assay the following tissues shall be analysed: liver, glandular stomach and duodenum.
   - the aneugenic potential of the Substance must be assessed by using a centromere staining technique if the substance induces an increase in the frequency of micronuclei in the OECD TG 474;
   - target tissue exposure must be demonstrated if the result of the OECD TG 474 is negative.

3. Hydrolysis as a function of pH (Annex VIII, Section 9.2.2.1.; test method: EU C.7./OECD TG 111)

The reasons for the request(s) are explained in Appendix 1.

Information required depends on your tonnage band

You must provide the information listed above for all REACH Annexes applicable to you in accordance with Articles 10(a) and 12(1) of REACH. The addressee of the decision and its corresponding information requirements based on registered tonnage band are listed in Appendix 3.

How to comply with your information requirements

To comply with your information requirements, you must submit the information requested by this decision in an updated registration dossier by the deadline indicated above. You must also **update the chemical safety report, where** relevant, including any changes to classification and labelling, based on the newly generated information.

You must follow the general requirements for testing and reporting new tests under REACH, see Appendix 4.

Appeal

This decision, when adopted under Article 51 of REACH, may be appealed to the Board of Appeal of ECHA within three months of its notification to you. Please refer to http://echa.europa.eu/regulations/appeals for further information.

Failure to comply

If you do not comply with the information required by this decision by the deadline indicated above, ECHA will notify the enforcement authorities of your Member State.

Authorised[1] under the authority of Mike Rasenberg, Director of Hazard Assessment

Appendix 1: Reasons for the request(s)
Appendix 2: Procedure
Appendix 3: Addressees of the decision and their individual information requirements
Appendix 4: Conducting and reporting new tests under REACH

[1] As this is an electronic document, it is not physically signed. This communication has been approved according to ECHA's internal decision-approval process.

Appendix 1: Reasons for the request(s)

Contents

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Reasons related to the information under Annex VII of REACH

1. In vivo mammalian alkaline comet assay combined with In vivo mammalian erythrocyte micronucleus test

1 Under Annex VII, Section 8.4., Column 2, an appropriate *in vivo* mammalian somatic cell genotoxicity study as referred to in Annex IX, point 8.4.4., must be performed in case of a positive result in any of the *in vitro* studies referred to in Annex VII, Section 8.4. The *in vivo* study must address the concerns raised by the *in vitro* study results, i.e. the chromosomal aberration concern or the gene mutation concern or both, as appropriate.

2 Your dossier contains positive results for the *in vitro* gene mutation study in bacteria (1979a; 1979b), *in vitro* chromosomal aberration test (1979c; 1979d) and *in vitro* gene mutation study in mammalian cells (1979e; 1980), which raise the concerns for gene mutations and chromosomal aberrations.

3 ECHA considers that the *in vivo* follow-up study is necessary to address the identified concern.

4 The information provided, its assessment and the specifications of the study design are addressed under Request 2.

Reasons related to the information under Annex VIII of REACH

2. In vivo mammalian alkaline comet assay combined with in vivo mammalian erythrocyte micronucleus test

5 Under Annex VIII, Section 8.4., Column 2, an appropriate *in vivo* mammalian somatic cell genotoxicity study as referred to in Annex IX, point 8.4., must be performed in case of a positive result in any of the *in vitro* studies referred to in Annex VII or VIII, Section 8.4. The *in vivo* study must address the concerns raised by the *in vitro* study results, i.e. the chromosomal aberration concern or the gene mutation concern or both, as appropriate.

2.1. Triggering of the information requirement

6 Your dossier contains positive results for the *in vitro* gene mutation study in bacteria (1979a; 1979b), *in vitro* chromosomal aberration test (1979c; 1979d) and *in vitro* gene mutation study in mammalian cells (1979e; 1980), which raise the concerns for gene mutations and chromosomal aberrations.

7 Therefore, the information requirement is triggered.

2.2. Information provided

8 ECHA understands that you have adapted this information requirement under on Column 2 based on germ cells studies and a carcinogenicity study based on which you have self-classified the Substance as Carc. 1B and applied risk management measures.

2.3. Assessment of the information provided

2.3.1. The provided adaptation does not meet the criteria of Annex VIII, Section 8.4., Column 2

9 Under Annex IX, Section 8.4., the study does not need to be conducted if the substance is known to cause germ cell mutagenicity, meeting the criteria for classification as germ cell mutagen category 1A or 1B, or to be a genotoxic carcinogen, meeting the criteria for classification both in the hazard class germ cell mutagenicity category 1A, 1B or 2 and in the hazard class carcinogenicity category 1A or 1B, and appropriate risk management measures are implemented.

10 The Substance is not classified as germ cell mutagen category 1A, 1B or 2.

11 Consequently, the criteria of Annex VIII, Section 8.4., Column 2 are not met and your adaptation is rejected.

12 ECHA considers that an appropriate *in vivo* follow up genetic toxicity study is necessary to address the concern(s) identified *in vitro*.

2.4. Test selection

13 The positive *in vitro* results available in the dossier indicate a concern for both chromosomal aberration and gene mutation.

14 The *in vivo* mammalian erythrocyte micronucleus test ("MN test", OECD TG 474) and the *in vivo* mammalian alkaline comet assay ("comet assay", OECD TG 489) can be combined in a single study (see OECD TG 474 paragraph 37c; OECD TG 489 paragraph 33; Guidance on IRs & CSA, Section R.7.7.6.3). While the MN test can detect both structural chromosomal

aberrations (clastogenicity) and numerical chromosomal aberrations (aneuploidy), the comet assay can detect primary DNA damage that may lead to gene mutations and/or structural chromosomal aberrations. A combined study will thus address both the identified concerns for chromosomal aberration as well as gene mutation.

15 The combined study, together with the results of the *in vitro* mutagenicity studies, can be used to make definitive conclusions about the mechanism(s) inducing *in vivo* mutagenicity and lack thereof. Furthermore, the combined study can help limit the number of tests performed and the number of animals used while addressing (structural and numerical) chromosomal aberrations as well as gene mutations.

16 You have submitted two germ cells studies that investigate mutations generally resulting from structural and/or numerical chromosomal aberrations. However, they do not investigate gene mutations and cannot be used to address the related concern. Moreover, these studies are germ cell tests and not somatic cell tests according to the OECD TG 474 or 475. For the first level of classification as germ cell mutagen, i.e. category 2 according to the CLP criteria, *in vivo* germ cell test results cannot be used and data obtained in somatic cells are necessary for that purpose.

17 Therefore, the comet assay combined with the MN test is the most appropriate study for the Substance.

18 In the comments to the draft decision, you agree to perform the requested study.

2.5. Specification of the study design

19 According to the test method OECD TG 489, rats are the preferred species. Other rodent species can be used if scientifically justified. According to the test method OECD TG 474, the test may be performed in mice or rats. Therefore, the combined study must be performed in rats, or if justified, in mice.

20 Having considered the anticipated routes of human exposure and adequate exposure of the target tissue(s) performance of the test by the oral route is appropriate.

21 In line with the test method OECD TG 489, the test must be performed by analysing tissues from liver as primary site of xenobiotic metabolism, glandular stomach and duodenum as sites of contact. 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.

22 According to the test method OECD TG 474, in order to demonstrate the ability of the study to identify clastogens and aneugens, you must include two concurrent positive controls, one known clastogen and one known aneugen (OECD TG 474, paragraph 25, Table 1).

23 The combination of the OECD TGs 489 and 474 should not impair the validity of and the results from each individual study. Careful consideration should be given to the dosing, and tissue sampling for the comet analysis alongside the requirements of tissue sampling for the mammalian erythrocyte micronucleus test (see OECD TG 489, e.g. Bowen et al. 2011 [1]).

[1] Bowen DE et al. (2011) Evaluation of a multi-endpoint assay in rats, combining the bone-marrow micronucleus test, the comet assay and the flow-cytometric peripheral blood micronucleus test. *Muta Res.*;722:7–19.

2.5.1. Assessment of aneugenicity potential

24 If the result of the *in vivo* MN test is positive, i.e. your Substance induces an increase in the frequency of micronuclei, you must assess the aneugenic potential of the Substance. In line with the OECD TG 474 (paragraph 42), you should use one of the centromere labelling or hybridisation procedures to determine whether the increase in the number of micronuclei is the result of clastogenic events (i.e. micronuclei contain chromosome fragment(s)) and/or aneugenic events (i.e. micronuclei contain whole chromosome(s)).

2.5.2. Investigation of target tissue exposure

25 The applicable test method OECD TG 474 states that "If there is evidence that the test substance(s), or its metabolite(s), will not reach the target tissue, it may not be appropriate to use this test". Additionally, a negative test result can be considered reliable only if "Bone marrow exposure to the test substance(s) occurred".

26 Therefore, to ensure that the data generated are adequate for hazard identification, you must take blood samples at appropriate times and measure plasma levels of the Substance and/or its metabolites (OECD TG 474, paragraph 40), unless exposure of the bone marrow can be demonstrated through other means, e.g. by showing a depression of immature to mature erythrocyte ratio (OECD TG 474, paragraph 48).

27 If the Substance is negative in this test, but it is not possible to demonstrate that bone marrow exposure to the Substance occurred, then ECHA will consider any remaining uncertainty concerning the mutagenic potential of the Substance and whether to request any further information.

2.5.3. Germ cells

28 You may consider collecting the male gonadal cells from the seminiferous tubules in addition to the other aforementioned tissues in the comet assay, as it would optimise the use of animals. You can prepare the slides for male gonadal cells and store them for up to 2 months, at room temperature, in dry conditions and protected from light. Following the generation and analysis of data on somatic cells in the comet assay, you should consider analysing the slides prepared with gonadal cells.

29 This type of evidence may be relevant for the overall assessment of possible germ cell mutagenicity including classification and labelling according to the CLP Regulation.

3. Hydrolysis as a function of pH

30 Hydrolysis as a function of pH is an information requirement under Annex VIII to REACH (Section 9.2.2.1.).

3.1. Information provided

31 You have provided:

(i) a hydrolysis study (2005) with the Substance;

3.2. Assessment of the information provided

3.2.1. The provided study does not meet the specifications of the test guideline(s)

32 To fulfil the information requirement, a study must comply with OECD TG 111 (Article 13(3) of REACH). This TG is designed as a tiered approach and each tier is triggered by the results of the previous tier. Therefore, the following specifications must be met:

33 Preliminary test (Tier 1)

a) the test must be conducted at least in duplicate at 50 ± 0.5°C for 5 days.

34 Hydrolysis testing (Tier 2)

b) the test is required if more than 10 % hydrolysis occurs after 5 days in the preliminary test (Tier 1);

c) the test must be performed at the pH value(s) at which the test material was found unstable in the preliminary test (i.e. > 10 % hydrolysis in Tier 1 test);

d) the test must be conducted at three temperatures, including the test temperature of 50°C.

35 Identification of hydrolysis products (Tier 3)

e) all major hydrolysis products observed in Tier 2 testing (i.e. at least those representing > 10% of the applied dose) must be identified using an appropriate analytical method (Tier 3).

36 Reporting

f) the test design is reported (e.g., number of replicates, type of test vessels, test duration);

g) the test conditions are reported (e.g., initial test material concentration, test temperature, pH values, buffers used);

h) the analytical method is described including appropriate information on performance parameters (i.e. specificity, recovery efficiency, precision, limits of determination (i.e. detection and quantification) and working range);

i) the proposed pathway of hydrolysis is reported.

37 In study (i):

38 Tier 1

a) the test was conducted at 25°C;

39 Tier 2 and 3

b) the preliminary test (Tier 1) indicates that at pH 4, 50 % hydrolysis occurs after 14.2 minutes and at pH 7, 50% hydrolysis occurs after 44.4 hours (1.85 days);

c) and d) further hydrolysis testing was performed at conditions that you consider to be physiologically relevant conditions (pH 1.2, 37°C). However, the test material was found unstable in the preliminary test (Tier 1) at pH 4 and pH 7, and hydrolysis testing (Tier 2) was not performed at these pH values; you do not report any testing done at 50°C.

e) major hydrolysis products (i.e. representing > 10% of applied dose) were not identified.

40 Reporting

f) the following pieces of information relevant to the test design are not reported: number of replicates, test duration;

g) the following pieces of information relevant to the test conditions are not reported: initial test material concentration, buffers used;

h) the analytical method used and its performance parameters are not reported;

i) no proposed pathway of hydrolysis has been reported.

41 Based on the above,

- there are critical methodological deficiencies resulting in the rejection of the study results. Specifically:
  - the percentage hydrolysis in the preliminary test requires to conduct a Tier 2 test at pH 4 and pH 7, however you have not performed hydrolysis testing (Tier 2); further, you have not identified the major hydrolysis products (Tier 3) and have not proposed a hydrolysis pathway;
- the reporting of the study is not sufficient to conduct an independent assessment of its reliability.
  - you have not reported pieces of information that are of key importance in interpreting the hydrolysis behaviour of the substance and assessing the reliability of the test, including information about the test design, the test conditions, and the analytical method used.

42 On this basis, the specifications of OECD TG 111 are not met.

43 Therefore, the information requirement is not fulfilled.

References

The following documents may have been cited in the decision.

Guidance on information requirements and chemical safety assessment (Guidance on IRs & CSA)

Chapter R.4 Evaluation of available information; ECHA (2011).
Chapter R.6 QSARs, read-across and grouping; ECHA (2008).
Appendix to Chapter R.6 for nanoforms; ECHA (2019).
Chapter R.7a Endpoint specific guidance, Sections R.7.1 – R.7.7; ECHA (2017).
Appendix to Chapter R.7a for nanomaterials; ECHA (2017).
Chapter R.7b Endpoint specific guidance, Sections R.7.8 – R.7.9; ECHA (2017).
Appendix to Chapter R.7b for nanomaterials; ECHA (2017).
Chapter R.7c Endpoint specific guidance, Sections R.7.10 – R.7.13; ECHA (2017).
Appendix to Chapter R.7a for nanomaterials; ECHA (2017).
Appendix R.7.13-2 Environmental risk assessment for metals and metal compounds; ECHA (2008).
Chapter R.11 PBT/vPvB assessment; ECHA (2017).
Chapter R.16 Environmental exposure assessment; ECHA (2016).

Guidance on data-sharing; ECHA (2017).
Guidance for monomers and polymers; ECHA (2012).
Guidance on intermediates; ECHA (2010).
All guidance documents are available online: https://echa.europa.eu/guidance-documents/guidance-on-reach

Read-across assessment framework (RAAF)

RAAF, 2017 Read-across assessment framework (RAAF); ECHA (2017).
RAAF UVCB, 2017 Read-across assessment framework (RAAF) – considerations on multi- constituent substances and UVCBs; ECHA (2017).

The RAAF and related documents are available online:
https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across

***OECD Guidance documents* (OECD GDs)**

OECD GD 23 Guidance document on aquatic toxicity testing of difficult substances and mixtures; No. 23 in the OECD series on testing and assessment, OECD (2019).
OECD GD 29 Guidance document on transformation/dissolution of metals and metal compounds in aqueous media; No. 29 in the OECD series on testing and assessment, OECD (2002).
OECD GD 150 Revised guidance document 150 on standardised test guidelines for evaluating chemicals for endocrine disruption; No. 150 in the OECD series on testing and assessment, OECD (2018).
OECD GD 151 Guidance document supporting OECD test guideline 443 on the extended one-generation reproductive toxicity test; No. 151 in the OECD series on testing and assessment, OECD (2013).

Appendix 2: Procedure

This decision does not prevent ECHA from initiating further compliance checks at a later stage on the registrations present.

ECHA followed the procedure detailed in Articles 50 and 51 of REACH.

The compliance check was initiated on 04 August 2022.

The deadline of the decision is set based on standard practice for carrying out OECD TG tests. It has been exceptionally extended by 12 months from the standard deadline granted by ECHA to take into account currently longer lead times in contract research organisations.

ECHA notified you of the draft decision and invited you to provide comments.

In your comments you agreed to the draft decision. ECHA took your comments into account and did not amend the request(s).

ECHA notified the draft decision to the competent authorities of the Member States for proposals for amendment.

As no amendments were proposed, ECHA adopted the decision under Article 51(3) of REACH.

Appendix 3: Addressee(s) of this decision and their corresponding information requirements

In accordance with Articles 10(a) and 12(1) of REACH, the information requirements for individual registrations are defined as follows:

- the information specified in Annex VII to REACH, for registration at 1-10 tonnes per year (tpa), or as a transported isolated intermediate in quantity above 1000 tpa;
- the information specified in Annexes VII and VIII to REACH, for registration at 10-100 tpa;
- the information specified in Annexes VII, VIII and IX to REACH, for registration at 100-1000 tpa;
- the information specified in Annexes VII to X to REACH, for registration at more than 1000 tpa.

Registrant Name	Registration number	Highest REACH Annex applicable to you
[redacted]	[redacted]	[redacted]

Where applicable, the name of a third party representative (TPR) may be displayed in the list of recipients whereas ECHA will send the decision to the actual registrant.

Appendix 4: Conducting and reporting new tests for REACH purposes

1. Requirements when conducting and reporting new tests for REACH purposes

1.1. Test methods, GLP requirements and reporting

(1) Under Article 13(3) of REACH, all new data generated as a result of this decision must be conducted according to the test methods laid down in a European Commission Regulation or to international test methods recognised by the Commission or ECHA as being appropriate.

(2) Under Article 13(4) of REACH, ecotoxicological and toxicological tests and analyses must be carried out according to the GLP principles (Directive 2004/10/EC) or other international standards recognised by the Commission or ECHA.

(3) Under Article 10(a)(vi) and (vii) of REACH, all new data generated as a result of this decision must be reported as study summaries, or as robust study summaries, if required under Annex I of REACH. See ECHA Practical Guide on How to report robust study summaries[2].

(4) Under the introductory part of Annexes VII/VIII/IX/X to REACH, where a test method offers flexibility in the study design, for example in relation to the choice of dose levels or concentrations, the chosen study design must ensure that the data generated are adequate for hazard identification and risk assessment.

1.2. Test material

(5) Selection of the Test material(s)

The Test Material used to generate the new data must be selected taking into account the following:

- the boundary composition(s) of the Substance,
- the impact of each constituent/ impurity on the test results for the endpoint to be assessed. For example, if a constituent/ impurity of the Substance is known to have an impact on (eco)toxicity, the selected Test Material must contain that constituent/ impurity.

(6) Information on the Test Material needed in the updated dossier

- You must report the composition of the Test Material selected for each study, under the "Test material information" section, for each respective endpoint study record in IUCLID.
- The reported composition must include all constituents of each Test Material and their concentration values.

With that detailed information, ECHA can confirm whether the Test Material is relevant for the Substance.

Technical instructions on how to report the above is available in the manual on How to prepare registration and PPORD dossiers (https://echa.europa.eu/manuals).

[2] https://echa.europa.eu/practical-guides