EUROPEAN CHEMICALS AGENCY

~~Confidential~~

Helsinki, 20 April 2021

Addressees
Registrant s of JS_Methylmethacrylate as listed in the last Appendix of this decision

Date of submission of the dossier subject to this decision
12/04/2013

Registered substance subject to this decision ("the Substance")
Substance name: Methyl methacrylate
EC number: 201-297-1
CAS number: 80-62-6

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 the deadline of ***26 July 2022***.

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

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

1. In vivo mammalian alkaline comet assay in rats, inhalation route, on the following tissues: liver and lung, (triggered by Annex VIII, Section 8.4., column 2)

B. Information required from all the Registrants subject to Annex IX of REACH

1. In vivo mammalian alkaline comet assay in rats, inhalation route, on the following tissues: liver and lung (Annex IX, Section 8.4., column 2)

Reasons for the request(s) are explained in the following appendices:

- Appendices entitled "Reasons to request information required under Annexes VIII to IX of REACH", respectively.

Information required depends on your tonnage band

You must provide the information listed above for all REACH Annexes applicable to you, and in accordance with Articles 10(a) and 12(1) of REACH:

- 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.

You are only required to share the costs of information that you must submit to fulfil your information requirements.

For certain endpoints, ECHA requests the same study from registrants at different tonnages. In such cases, only the reasoning why the information is required at lower tonnages is provided in the corresponding Appendices. For the tonnage where the study is a standard information requirement, the full reasoning for the request including study design is given. Only one study is to be conducted; the registrants concerned must make every effort to reach an agreement as to who is to carry out the study on behalf of the other registrants under Article 53 of REACH.

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 testing and reporting requirements provided under the Appendix entitled "Requirements to fulfil when conducting and reporting new tests for REACH purposes". In addition, you should follow the general recommendations provided under the Appendix entitled "General recommendations when conducting and reporting new tests for REACH purposes". For references used in this decision, please consult the Appendix entitled "List of references".

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 Christel Schilliger-Musset, Director of Hazard Assessment

[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 A: Reasons to request information required under Annex VIII of REACH

1. In vivo mammalian alkaline comet assay

Under Annex VIII, Section 8.4, column 2 of REACH, the performance of an appropriate *in vivo* somatic cell genotoxicity study must be considered if there is a positive result in any of the *in vitro* genotoxicity studies in Annex VII or VIII.

The ECHA guidance R.7a states that following a positive result in an *in vitro* test, "*adequately conducted somatic cell in vivo testing is required to ascertain if this potential can be expressed in vivo. In cases where it can be sufficiently deduced that a positive in vitro finding is not relevant for in vivo situations (e.g. due to the effect of the test substances on pH or cell viability, in vitro-specific metabolism: see also Section R.7.7.4.1), or where a clear threshold mechanism coming into play only at high concentrations that will not be reached in vivo has been identified (e.g. damage to non-DNA targets at high concentrations), in vivo testing will not be necessary.*"

Your dossier contains positive results for the *in vitro* cytogenicity tests and the *in vitro* gene mutation studies in mammalian cells which raise the concern for chromosomal aberration and gene mutation.

ECHA considers that an appropriate *in vivo* follow up mutagenicity study is necessary to address the concern identified *in vitro*.

The assessment of the information provided to fulfil this information requirement, the selection of the requested test and the test design are addressed in request B.1.

Appendix B: Reasons to request information required under Annex IX of REACH

1. In vivo mammalian alkaline comet assay

Under Annex IX, Section 8.4, column 2 of REACH, the information requirement for an appropriate *in vivo* somatic cell genotoxicity study is triggered if 1) there is a positive result in any of the *in vitro* genotoxicity studies in Annex VII or VIII and 2) there are no appropriate results already available from an *in vivo* somatic cell genotoxicity study.

In relation to the first condition, your dossier contains positive results for the *in vitro* cytogenicity tests and the *in vitro* gene mutation studies in mammalian cells which raise the concern for chromosomal aberration and gene mutation. In the dossier you state that "*from mammalian cell culture assays it may be concluded that methyl methacrylate is a high toxicity clastogen (i.e. induction of chromosomal aberrations is bound to highly toxic doses). These findings are in line with results from mouse lymphoma assays where positive findings seem to be due to the induction of small colonies*", supporting the concern for clastogenicity. Therefore the the concern for chromosomal aberrations appears to be more clearly established than the one for gene mutations.

In relation to the second condition, you have provided data from the following sources of information as part of an adaptation under section 1.2 of Annex XI (weight of evidence):

i. [redacted] (1986), similar to OECD TG 478 (Rodent Dominant Lethal Test) with the Substance
ii. [redacted] (1976), similar to OECD TG 475 (Mammalian Bone Marrow Chromosome Aberration Test) with the Substance
iii. [redacted] (1979), similar to OECD TG 475 (Mammalian Bone Marrow Chromosome Aberration Test) with the Substance
iv. Hachiya (1982), similar to OECD TG 474 (Mammalian Erythrocyte Micronucleus Test) with the Substance
v. [redacted] (1994), reference of a publication: Absence of Mutagenicity in Peripheral Lymphocytes of Workers Occupationally Exposed to Methyl Methacrylate (study investigates sister chromatid exchange frequency and chromosome aberrations in male workers occupationally exposed to the Substance)
vi. Bagramyan (1974), chromosome aberration assay, no test guideline
vii. Ouyang (1989), (no information provided on the type of assay performed)
viii. Fedyukovich (1981), "chromosome aberration assay", no test guideline
ix. Fedyukovich (1991), "chromosome aberration assay", no test guideline,
x. Jensen (1991), "*as OECD 474*",

Annex XI, Section 1.2 states that there may be sufficient weight of evidence from several independent sources of information leading to assumption/conclusion that a substance has or has not a particular dangerous (hazardous) property, while information from a single source alone is insufficient to support this notion.

According to ECHA Guidance R.4, a weight of evidence adaptation involves an assessment of the relative values/weights of the different sources of information submitted. The weight given is based on the reliability of the data, consistency of results/data, nature and severity of effects, and relevance and coverage of the information for the given regulatory information requirement. Subsequently, relevance, reliability, coverage, consistency and results of these sources of information must be balanced in order to decide whether they together provide sufficient weight to conclude that the Substance has or has not the (dangerous) property investigated by the required study.

Annex XI, section 1.2 requires that adequate and reliable documentation is provided to describe your weight of evidence approach.

However, you have not submitted any explanation why the sources of information provide sufficient weight of evidence leading to the conclusion/assumption that the Substance has or has not a particular dangerous property.

In spite of this critical deficiency, ECHA has nevertheless assessed the validity of your adaptation and identified the following issues.

To fulfil the information requirement, an *in vivo* somatic cell study that addresses the concern for chromosomal aberration is required. ECHA Guidance R.7.7.3.1. clarifies that the *in vivo* study must be either a micronucleus test or a chromosomal aberration test, performed according to OECD TG 474 or 475, respectively[2]. Alternatively, the *in vivo* mammalian alkaline comet assay ("comet assay", OECD TG 489) is a suitable test to be performed.

Based on the information provided in the dossier, due to the rapid metabolism of the Substance (MMA and the other methacrylate esters are readily absorbed by all routes and rapidly hydrolyzed by carboxylesterases to methacrylic acid (MAA) and the respective alcohol), there is a concern for chromosomal aberrations in the initial site of contact tissues, which cannot be evaluated by performing an OECD TG 474/475, since these studies only measure effects in the bone marrow (distant tissue). The comet assay is suitable to follow up the positive *in vitro* result for gene mutations and chromosomal aberrations. Moreover, it enables the generation of information regarding potential genotoxic effects at the site of contact. Therefore, the *in vivo* comet assay is the most appropriate follow-up test for the Substance.

The comet assay (OECD TG 489) investigates if the substance cause:
(1) primary DNA damage that could lead to gene mutations and/or chromosomal aberrations in somatic cells of animals (usually rodents), and
(2) DNA damage in the tissues at first site of contact, for example stomach after oral exposure and lungs after inhalation exposure.

(1) Chromosomal aberrations in somatic cells of animals (usually rodents)

Source of information (i.) does not investigate chromosomal aberrations in somatic cells but in germ cells. Therefore, this study does not provide information that would contribute to the conclusion on the information investigated by the required study.

Sources of information (vii.) does not investigate chromosomal aberrations in somatic cells of animals because it does not provide information on the type of assay performed.

The sources of information ii. to vi., viii. to x., provide relevant information on chromosomal aberrations in somatic cells of animals.

However, the reliability of these sources of information is significantly affected by the following deficiencies:

i. Studies ii. to iv
The specifications/conditions of OECD TG 474 or 475 include: (a) the collection of bone marrow at two different times after single treatment; (b) the scoring of at least 4000 immature erythrocytes per animal (OECD TG 474) / the analysis of at least 200

[2] ECHA Guidance R.7a, Table R.7.7-3, p.558

metaphases for each animal (OECD TG 475).

However, the reported data for studies ii. to iv. indicates that (a) the bone marrow was collected only 24 hours after single treatment; (b) only 2000 erythrocytes were evaluated per animal (OECD TG 474) / only 50 cells per animal were analysed (OECD TG 475).

As indicated in OECD TG 474/475, this information is required to determine the acceptability of the test. As explained above, the acceptability criteria are not fulfilled. Therefore the test results, provided in studies ii. to iv., cannot be considered as a reliable source of information that could contribute to the conclusion on chromosomal aberration in somatic cells investigated by the required study.

ii. Studies v. to x.
Pursuant to Article 10(a)(vii) of the REACH Regulation, the information set out in Annex VII to XI must be provided in the form of a robust study summary. Article 3(28) defines a robust study summary as a detailed summary of the objectives, methods, results and conclusions of a full study report providing sufficient information to make an independent assessment of the study minimising the need to consult the full study report.

However, for the sources of information v. to x. you have not provided adequate and reliable documentation in a form of a robust study summary, as required by Article 10(a)(vii) and Article 3(28).

(2) Investigation of first site of contact tissues

None of the sources of information provided investigate DNA damage in first site of contact tissues and consequently they do not provide information that would contribute to the conclusion on the information investigated by OECD TG 489.

Taken together, even if the sources of information (ii. to vi., viii. to x.) provide information on chromosomal aberrations in somatic cells in animals, their reliability is affected so significantly that they cannot be taken into consideration in a weight of evidence approach. Moreover, none of these sources of information provide information on tissues of first site contact.

Therefore, it is not possible to conclude, based on any source of information alone or considered together, whether your Substance has or has not the particular dangerous property foreseen to be investigated in an OECD TG 489. Therefore, your adaptation is rejected.

In your comments on the draft decision, you agreed that the dossier of the Substance contains positive results for the *in vitro* cytogenicity tests and the *in vitro* gene mutation studies in mammalian cells. However, you argue that almost all of the weak positive results in these *in vitro* studies were reached in cytotoxic concentrations leading to your assessment that the Substance "is a high toxicity clastogen (i.e. induction of chromosomal aberrations is bound to highly toxic doses)". In addition, you state that the observations were done in pre-guideline studies with various deviations when compared against the current test guidelines. Therefore, you intend to perform a new set of relevant *in vitro* guideline compliant studies and assess them together with the existing pre-guideline studies in a valid weight of evidence approach (WoE) first before a decision is made on further animal testing.

ECHA acknowledges your intention to fulfil the information requirement with new *in vitro* mutagenicity studies and an updated weight of evidence (WoE) justification. You may, under your own responsibility, carry out your testing programme. If the resulting data does not support your intended updated weight of evidence hypothesis, you remain responsible for complying with this decision by the set deadline.

Currently, the *in vitro* data available in your dossier, provided under a weight of evidence adaptation, indicate a concern for chromosomal aberration. The conditions set out in Annex IX, Section 8.4, column 2 are therefore met and the information requirement for an appropriate *in vivo* somatic cell genotoxicity study is triggered.

Consequently, the information provided in your dossier and your comments on the draft decision is not sufficient to fulfil the information requirement.

A. Test selection

As indicated above the *in vivo* comet assay is the most appropriate follow-up test for the Substance.

B. Test design

According to the test method OECD TG 489, the test must be performed in rats. Having considered the anticipated routes of human exposure and the need for adequate exposure of the target tissue(s) performance of the test by the inhalation route is appropriate. The inhalation route is specified as a relevant route of administration, as lungs are a sensitive tissue and can be reached more quickly by this substance than the intestines, and the parenteral route is not relevant for human risk assessment of industrial chemicals.

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 and lung as sites of contact.

C. Germ cells

A subsequent germ cell genotoxicity study (TGR/OECD TG 488, or CA on spermatogonia/OECD TG 483) may still be required under Annex IX of REACH, in case 1) an *in vivo* genotoxicity test on somatic cell is positive, and 2) no clear conclusion can be made on germ cell mutagenicity.

Therefore, you may consider to collect 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, in accordance to Annex IX, Section 8.4., column 2, you should consider analysing the slides prepared with gonadal cells. 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.

Appendix C: Requirements to fulfil when conducting and reporting new tests for REACH purposes

A. 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[3].

B. Test material

Before generating new data, you must agree within the joint submission on the chemical composition of the material to be tested (Test Material) which must be relevant for all the registrants of the Substance.

1. Selection of the Test material(s)

   The Test Material used to generate the new data must be selected taking into account the following:
   - the variation in compositions reported by all members of the joint submission,
   - 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.

2. 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 and other parameters relevant for the property to be tested.

This information is needed to assess whether the Test Material is relevant for the Substance and whether it is suitable for use by all members of the joint submission.

Technical instructions on how to report the above is available in the manual on How to prepare registration and PPORD dossiers[4].

[3] https://echa.europa.eu/practical-guides
[4] https://echa.europa.eu/manuals

Appendix D: 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 07 August 2019.

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

ECHA took into account your comments 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 E: List of references - ECHA Guidance[5] and other supporting documents

Evaluation of available information
Guidance on information requirements and chemical safety assessment, Chapter R.4 (version 1.1., December 2011), referred to as ECHA Guidance R.4 where relevant.

QSARs, read-across and grouping
Guidance on information requirements and chemical safety assessment, Chapter R.6 (version 1.0, May 2008), referred to as ECHA Guidance R.6 where relevant.

Read-across assessment framework (RAAF, March 2017)[6]

RAAF - considerations on multiconstituent substances and UVCBs (RAAF UVCB, March 2017)[6]

Physical-chemical properties
Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Toxicology
Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7c (version 3.0, June 2017), referred to as ECHA Guidance R.7c in this decision.

Environmental toxicology and fate
Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7b (version 4.0, June 2017), referred to as ECHA Guidance R.7b in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7c (version 3.0, June 2017), referred to as ECHA Guidance R.7c in this decision.

PBT assessment
Guidance on information requirements and chemical safety assessment, Chapter R.11 (version 3.0, June 2017), referred to as ECHA Guidance R.11 in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.16 (version 3.0, February 2016), referred to as ECHA Guidance R.16 in this decision.

Data sharing
Guidance on data-sharing (version 3.1, January 2017), referred to as ECHA Guidance on data sharing in this decision.

OECD Guidance documents[7]

[5] https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment
[6] https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across
[7] http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm

Guidance Document on aqueous-phase aquatic toxicity testing of difficult test chemicals – No 23, referred to as OECD GD 23.

Guidance document on transformation/dissolution of metals and metal compounds in aqueous media – No 29, referred to as OECD GD 29.

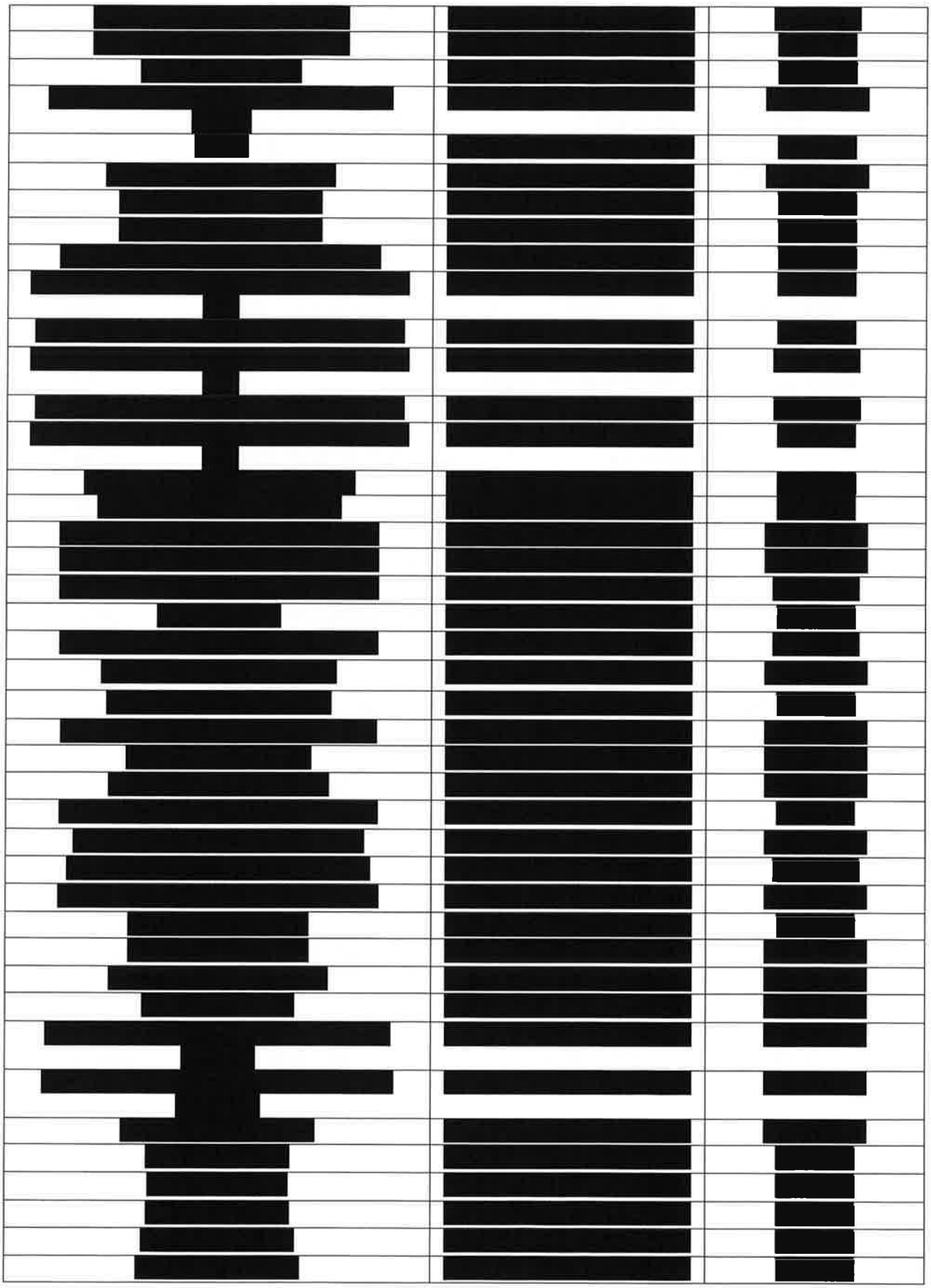
Guidance Document on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption – No 150, referred to as OECD GD 150.

Guidance Document supporting OECD test guideline 443 on the extended one-generation reproductive toxicity test – No 151, referred to as OECD GD 151.

Appendix F: Addressees of this decision and the corresponding information requirements applicable to them

You must provide the information requested in this decision for all REACH Annexes applicable to you.

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.