Nordic Council of Ministers

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Chemical induced effects on the developing nervous and immune systems

Protection against the harmful effects of chemicals on the development of the nervous and immune system, through better use of already planned animal studies

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[1] The information from Appendices I, II and IV regarding relevant data obtained through the conducted open literature search, all assessed chemicals and identified metabolites of the assessed substances using the OECD Toolbox program respectively, was not made available at the time this report was published due to confidentiality issues.

Foreword

This project was conducted with funding from the Nordic Council of Ministers. The work was performed by the project consultant, Giorgiana-Adina Tenie (M.Sc. Toxicology; B.Pharm), and guided by the steering group (Table 1). The information gathered in this project is being further assessed by the Danish Environmental Protection Agency in collaboration with Competent Authorities from other Member States. When adequate and relevant, the obtained information has already been for some substances and will be used in the future cases to propose inclusion of examination of effects on the developing immune system and/or developing nervous system in the Extended One Generation Reproductive Toxicity Studies (EOGRTS) proposed to be performed as part of the REACH standard information requirements. Some of the proposals have already been brought forward and discussed at the Member State Committee in ECHA.

This work is relevant for authorities in the EU (member states, ECHA and the EU Commission), and for authorities from other countries across the world, which can make use of the results. From a broader perspective, the target group of this project is consumers, professional and industrial workers, since the work aims to improve protection from adverse effects on the developing nervous and immune system.

Table 1. Members of the steering group of the NKG project

Name	Agency	Country
Marie Louise Holmer	Danish Environmental Protection Agency	Denmark
Katarzyna Malkiewicz	Swedish Chemicals Agency	Sweden
Joop de Knecht	National Institute for Public Health and the Environment	The Netherlands
Tialda Bouwman	National Institute for Public Health and the Environment	The Netherlands
Cecile Michel	French Agency for Food, Environmental and Occupational Health & Safety	France
Ann-Karin Olsen	Norwegian Institute of Public Health	Norway

Stockholm, February 2017

Giorgiana-Adina Tenie,
Consultant

Abbreviation list

CAS	Chemical Abstract Service
CCH	Compliance Check(s)
CLP	Classification, Labelling and Packaging of substances and mixtures
DA	Dopamine
DD	Draft Decision
DEV	Dossier Evaluation
DNT	Developmental Neurotoxicity
DIT	Developmental Immunotoxicity
EC	European Commission
ECHA	European Chemicals Agency
ED	Endocrine Disrupting / Disruptors
EOGRTS	Extended One Generation Reproductive Toxicity Study
EU	European Union
F2	Second generation
GABA	Gamma aminobutyric acid
HSDB	Hazardous Substances Data Base
MoA	Mode of Action
MSCA(s)	Member State Competent Authority / Authorities
MSC	Member State Committee
NTP	National Toxicology Program
NKG	Nordiska Kemikaliegruppen/ Nordic Chemicals Group
OECD	Organization for Economic Co-operation and Development
PfA(s) / PFA	Proposal(s) for Amendment
(Q)SAR	Quantitative Structure Activity Relationship
RA	Risk Assessment
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
REG	Registrant(s)
ROS	Reactive Oxygen Species
SIN	Substitute it Now! (Chemsec)

SEV	Substance Evaluation
TEDX	The Endocrine Disruption Exchange
TG	Test Guideline
TPE	Testing Proposal Examination
UVCB	Substances of unknown or variable composition, complex reaction products or biological materials

1. Background

Effects of chemicals on the developing brain and immune system are of high concern in the Nordic countries and the EU. Such effects can be caused by substances acting through many different modes of action, including endocrine disrupting substances (for example bisphenol A affects the developing brain and the immune system, and perflourinated substances also interfere with the immune system).

Information on developmental neurotoxicity (DNT) and developmental immunotoxicity (DIT) is not available for most of the substances used for instance in consumer products today, but under current EU regulation (REACH) there is an unique possibility to get information on these effects for some of the substances used in high tonnages. The testing for DNT and DIT is concern driven and it is therefore crucial to identify all relevant data to trigger these investigations.

Under REACH, the requirements in regards to testing for reproductive toxicity depends on the production (or import) tonnage level of the substance. For the highest tonnage levels, an Extended One Generation Reproductive Toxicity Study (EOGRTS) (OECD TG 443) has recently substituted the “two generation reproductive toxicity study” (OECD TG 416) as a standard information requirement.

The EOGRTS was implemented in REACH in 2015. The European Chemicals Agency (ECHA) can therefore now in the evaluation processes (including dossier evaluation: compliance check and testing proposals, and substance evaluation, see section 1.1) request EOGRTS. In each case, the design of the study must be specified. The EOGRTS in REACH is composed of a basic design, which can be supplemented with investigation of the following areas (cohorts): Second generation (F2), effects on the development of the brain (DNT) and effects on the immune system during development (DIT) – if data are available, which trigger these cohorts (see section 1.2). Such conditional testing makes the availability of relevant triggering data crucial for justifying the mentioned investigations.

In the dossier evaluation process, if an EOGRTS is considered, ECHA searches for data relevant for triggering of the cohorts in the registration dossier and in a limited number of publicly available databases. ECHA’s search strategy includes identification of analogues used by the Registrants or assessed by international evaluation bodies, but does not include search in the open literature [ECHA, 2016a]. Searching for triggers in the public literature is therefore either left out or a possibility for the Member State Competent Authorities MSCAs to perform.

From 2016, some MSCAs e.g. from Denmark and Sweden, assisted ECHA in a search for triggers outside the registration dossiers. This included a project (for a master degree) in which information in the open literature for potential triggers for 40 substances was searched and data for about 20% of substances found. It was at that time acknowledged that the resources in the national agencies are very limited, and it would not be possible to carry out a search for around 400 pending cases.
This Nordic project was therefore initiated to promote inclusion of the relevant cohorts when the EOGRTS was requested.

1.1. Evaluation process in REACH

The Evaluation process under REACH (Figure 1) consists of Dossier Evaluation (DEV) and Substance Evaluation [ECHA, 2017] (SEV). Dossier Evaluation (DEV) aims to improve quality of the registration dossiers and it includes two processes: Testing Proposal Examinations (TPE) and Compliance Checks (CCH), while SEV aims to clarify identified concern(s) [ECHA, 2017].

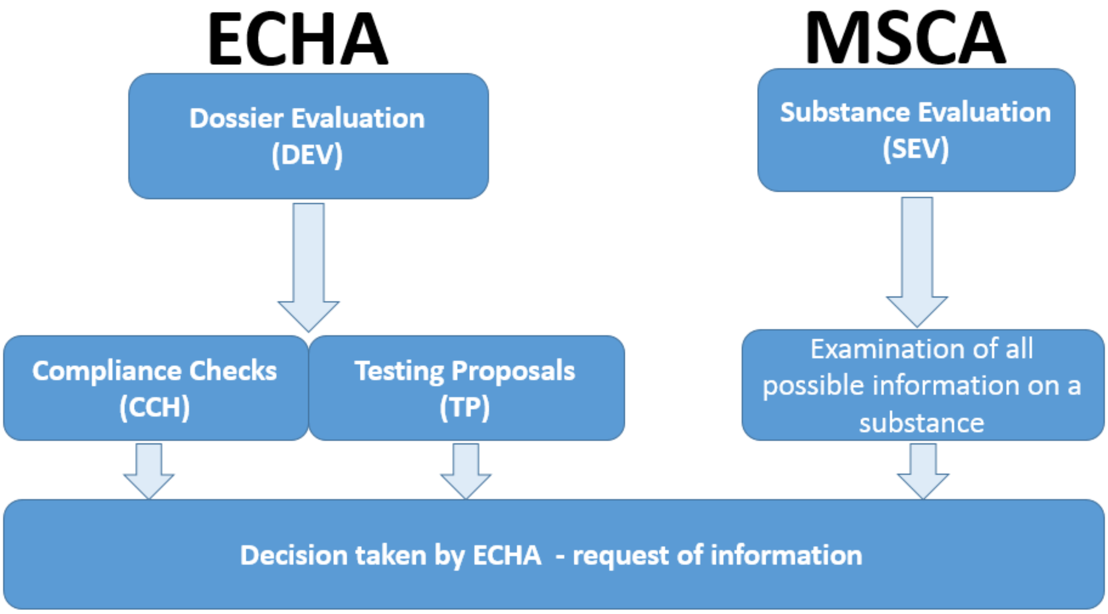
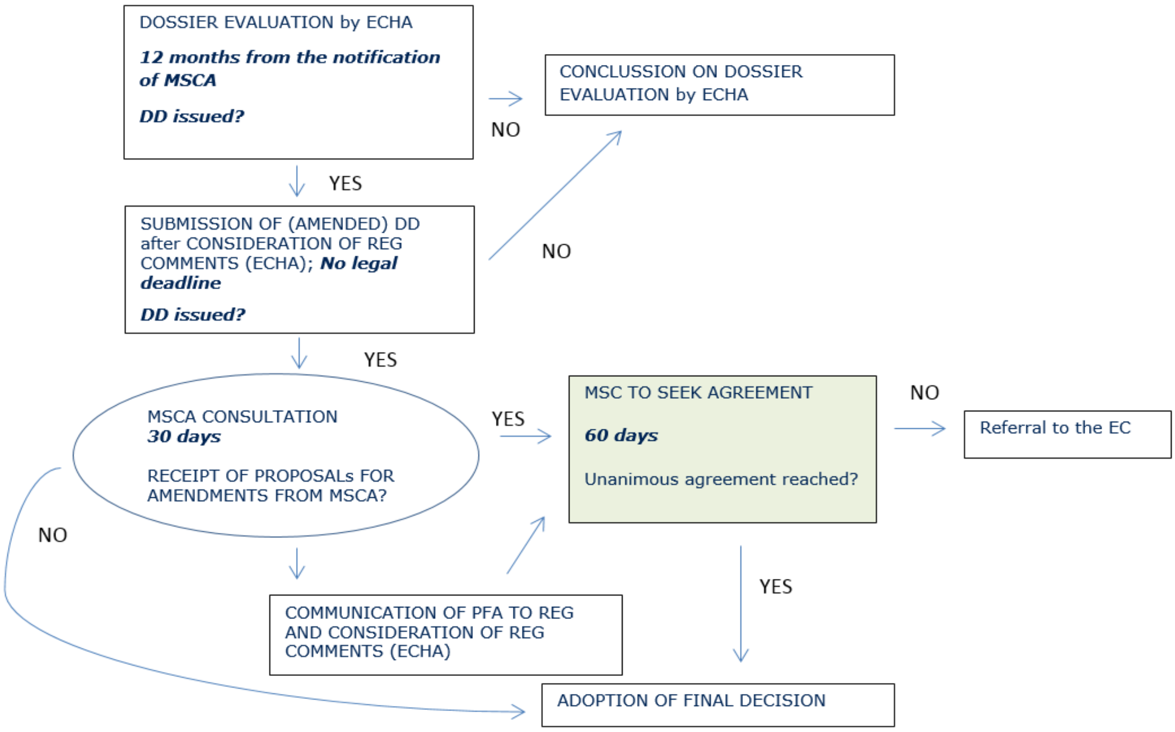


Figure 1. Evaluation process in REACH
(Modified from https://echa.europa.eu/regulations/reach/evaluation/evaluation-procedure).

The process, starting from initial ECHA evaluation until final decision on the requested test, involves different stakeholders i.e. ECHA, Member States Competent Authorities, Industry/Registrant(s), Member State Committees (MSC) and European Commission (EC) (see Flowchart 1).



Flowchart 1. Decision making on Dossier Evaluation Draft Decisions [ECHA, 2017].

1.2. EOGRTS design

EOGRTS (OECD 443) has a modular design, and the "basic" design is a standard requirement in REACH, while inclusion of DNT, DIT and F2 cohorts is conditional. The criteria for the inclusion of DNT, DIT and F2 are defined in REACH (see below) and those so called "triggers"[2] are further elaborated in the guidance document [ECHA, 2015] .

REACH Section 8.7.3 Annex IX and X:

*"(…) **developmental neurotoxicity and/or (…) developmental immunotoxicity shall be proposed** (…), in case of particular concerns on (developmental) neurotoxicity or (developmental) immunotoxicity justified by any of the following:*
— existing information on the substance itself derived from relevant available in vivo or non-animal approaches (e.g. abnormalities of the CNS, evidence of adverse effects on the nervous or immune system in studies on adult animals or animals exposed prenatally), or

[2] Definition of a "trigger" - Triggers are findings which challenge the existing toxicity database. This means that due to existing triggers it is not possible to conclude on the potential for adverse health effects for a substance, and to address the concern further information may be needed or is needed, depending on the condition. The term "triggers" is used here as a general term instead of various other possible terms (such as an alert, condition, indication, indication of concern, serious concern, a particular concern) [ECHA, 2015].

— specific mechanisms/modes of action of the substance with an association to (developmental) neurotoxicity and/or (developmental) immunotoxicity (e.g. cholinesterase inhibition or relevant changes in thyroidal hormone levels associated to adverse effects), or
— existing information on effects caused by substances structurally analogous to the substance being studied, suggesting such effects or mechanisms/modes of action. "

"(…) F2 generation shall be proposed (…) if:
(a) the substance has uses leading to significant exposure of consumers or professionals, taking into account, inter alia, consumer exposure from articles, and
(b) any of the following conditions are met:
— the substance displays genotoxic effects in somatic cell mutagenicity tests in vivo which could lead to classifying it as Mutagen Category 2, or
— there are indications that the internal dose for the substance and/or any of its metabolites will reach a steady state in the test animals only after an extended exposure, or
— there are indications of one or more relevant modes of action related to endocrine disruption from available in vivo studies or non-animal approaches. "

2. Purpose and objective of the project

In this project, the Nordic Countries have assisted ECHA in searching for information relevant for triggering of DNT, DIT outside the registration dossiers. The aims were to promote design of EOGRTS with specific investigations DNT, DIT and F2 when justified by the evidence found. The overall aim of the project was to increase the level of protection of the population from effects on the developing brain and the developing immune system. Information on endocrine effects or mode of action has been considered as a trigger for all three cohorts DNT, DIT and F2. The project also aimed to support a better utilization of animal studies, since when cohorts are investigated, more information is obtained from the animals already used in the studies.

The project objective was to search the open scientific literature for EOGRTS triggers for approximately 100 substances. Secondly, for a limited number of cases, an analysis of the outcome from the regulatory decision process in respect to the design of the EOGRTS was included in the project.

3. Methodology

3.1. Data search

Search strategy

The search strategy used in this project was developed based on the text regarding inclusion of the cohorts in the EOGRTS from the standard data requirement for EORGTS according to REACH Annexes IX and X, Section 8.7.3. (see section 1.2), as well as the ECHA endpoint specific guidance chapter R.7.6 of ECHA, TGD R.7a [ECHA, 2015]. The search strategy was subject to refinement during the project. Therefore, some searches were performed for all substances, whereas others were only performed for the last app. 50 substances, as outlined below:

A) Search in the open scientific literature using PubMed (all substances) and also PubChem, HSDB, NTP and Haz-Map (for app. 50 substances).

B) Search in publicly available databases on suspected endocrine disruptors (all substances).

C) Search for (Q)SAR predictions (all substances).

D) Search for the above (A, B, C) for constituents in cases where registered substances were multiconstituent substances or UVCBs.

E) Search for the above (A, B, C) for analogues identified by ECHA (all substances).

F) Search for possible triggering information in the repeated dose toxicity and reproductive toxicity studies in the registration dossier (for app. 50 substances).

G) Search for metabolites (for app. 50 substances)

Ad A)

The scientific literature search (screening) for all substances was performed using PubMed as a primary database. To identify potential triggers for extending the EOGRTS design to include the F2, DNT and/or DIT cohorts, specific filters in association with the name of the assessed substance (parent chemical) were employed (Table 2). In addition, synonyms and trade names of the assessed substances according to the registration dossier, were used in combination with the search filters.

Table 2. Specific search filters used in the literature screening process, based on ECHA, 2015, pages 408-417.

Search filters
Endocrine, Hormon*, Ovar*, Ovary, Testes, Testis, Uterus, Cervix, Epididym*, Seminal, Coagulating, Prostat*, Prostate, Vagin*, Pituitary, Mammary, Anogenital, Nipple, Steroi*, Aromatase, Gestation, Neurotox*, Nervous, Spinal, Brain, Nerves, Neurite, Neuronal, Synaptogenesis, Neuropathy, Cholinesterase, Gait, Narcosis, Seizures, Thyr*, Thyroid, Estro*, Oestro*, Antiestro*, Andro*, Androgenic, Antiandro*, Immunology, Immunotoxic, Thymus, White blood cells, Leucocytes, Spleen, NK-cell, Phagocytosis, Oxidative burst, Acquired immunity, Immunological memory, T-cells, Antibody production, Sensitization.

The screening process was refined during the project through extending the number of the used databases. In order to allow a more extensive search of the publicly available scientific literature for potential triggers for the F2, DNT and/or DIT cohorts, in addition to screening only PubMed (for the first investigated 50 substances), 4 more databases were used (for the last app. 50 substances):

- ✓ PubChem, available at https://pubchem.ncbi.nlm.nih.gov/
- ✓ HSDB, available at https://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB
- ✓ NTP, available at https://ntpsearch.niehs.nih.gov/
- ✓ Haz-Map, available at https://hazmap.nlm.nih.gov/

Ad B)

Three different publicly available online sources were used for investigating the ED potential (ED-screening) of the assessed substances:

- ✓ TEDX list of endocrine disruptors, available at http://endocrinedisruption.org/
- ✓ SIN list, available at http://chemsec.org
- ✓ EU Commission database of suspected endocrine disruptors, available at http://ec.europa.eu/environment/chemicals/endocrine/strategy/substancesen.html

Ad C)

(Q)SAR predictions of endocrine disruptive properties were obtained from the Danish (Q)SAR database, and only the positive predictions were used as possible triggering elements for further assessment:

- ✓ Danish (Q)SAR database: http://qsardb.food.dtu.dk/db/index.html

Ad D)

The analogues of each parent chemical identified by ECHA in their search was noted, and the open literature, databases on suspected endocrine disruptors and (Q)SAR predictions were further screened for information on each analogue (as described above, in Ad A, B and C).

Ad E)

Some of the assessed chemical substances were substances of unknown or variable composition, complex reaction products or biological materials, collectively called UVCBs under the REACH legislation. In these cases, the constituents of each UVCB were identified from the registration dossier and the searches described above (in A, B, C) were performed for each of the found constituents.

Ad F)

Another refinement in the search methodology introduced during the project, was the inclusion of the existing reproductive toxicological information (from the registration dossier) for each parent chemical, in the Microsoft Excel document used to collect the results. This contributed to directing the search of the open literature towards other unknown / not reported DNT and / or DIT-related effects. Therefore, the screening process became less time-consuming through not focusing on the references related to already known and reported DNT and/or DIT triggers.

Ad G)

The OECD QSAR Toolbox program was employed for identifying the metabolites of the parent chemicals. Two different types of metabolites, documented (observed) and simulated, were retrieved for each assessed substance (Appendix III).

Since it was the case that one parent chemical had several metabolites, the results of this step were compiled in a different Microsoft Excel document than the Microsoft Excel one with the results of the project (Appendix IV). Due to a limited timeframe for conducting this Nordic project, it was not possible to perform the literature search process for the retrieved metabolites.

3.2. Assessed substances

The substances included in the project consisted of: 121 “parent” substances, 46 constituents of the parent substances and 82 analogues (Appendix II).

A total of 385 metabolites were identified (using the OECD QSAR Toolbox program) for 35 of the 121 parent chemicals (Appendix IV) but the literature screening was not conducted for the metabolites, due to time restraints.

3.3. Collection of information on substances

The scientific articles retrieved from PubMed and other databases were further saved in pdf format and assessed for potential to trigger the F2, DNT and/or DIT cohorts.

All obtained information was recorded in Microsoft Excel documents. This information was forwarded to the members of the steering group (see Table 1).

3.4. Analysis of the outcome from the Member State Committee

For substances for which all available information (gathered in this project or otherwise present in the registration dossiers and/or provided by ECHA) raised a concern for DNT and/or DIT, and the cohorts were not already included in the draft decision proposed by ECHA, the MSCAs submitted proposals to include the relevant cohorts. The decision making for such cases was referred to MSC.

During the timeline of this project, the outcome of five MSC meetings (MSC 47 – MSC 51) for 19 substances was evaluated using the publicly available "Minutes" from those meetings, where the summary records of MSC discussions and voting are included. Detailed information on the content of PfAs, the responses from ECHA and MSC discussions are not publicly available. 14 substances with EOGRTS included in the agenda of MSC 52 are listed but the outcome not analysed because the Minutes from this meeting were not available at the time this report was drafted.

4. Results

4.1. Relevant data obtained in the conducted search

Data obtained through the literature search

The literature screening process retrieved possible relevant references for 84 out of 249 assessed substances (parent chemicals, analogues, constituents), for 48 out of 121 parent chemicals, for 26 out of 82 investigated analogues and for 10 out of 46 constituents (Figure 2).

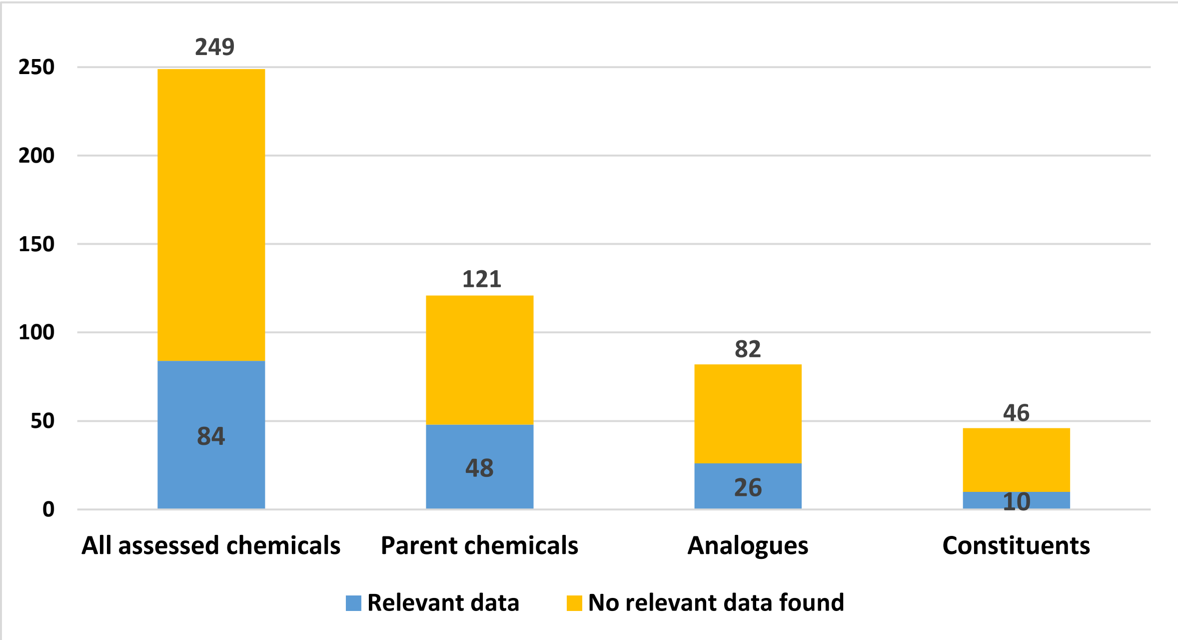


Figure 2. Statistical information on data obtained through the literature search process.

The retrieved relevant references were linked to DNT for 28 cases, DIT in 29 and 14 ED MoA (Figure 3), the latter with data relevant for potential triggering of all three cohorts DNT, DIT and F2.

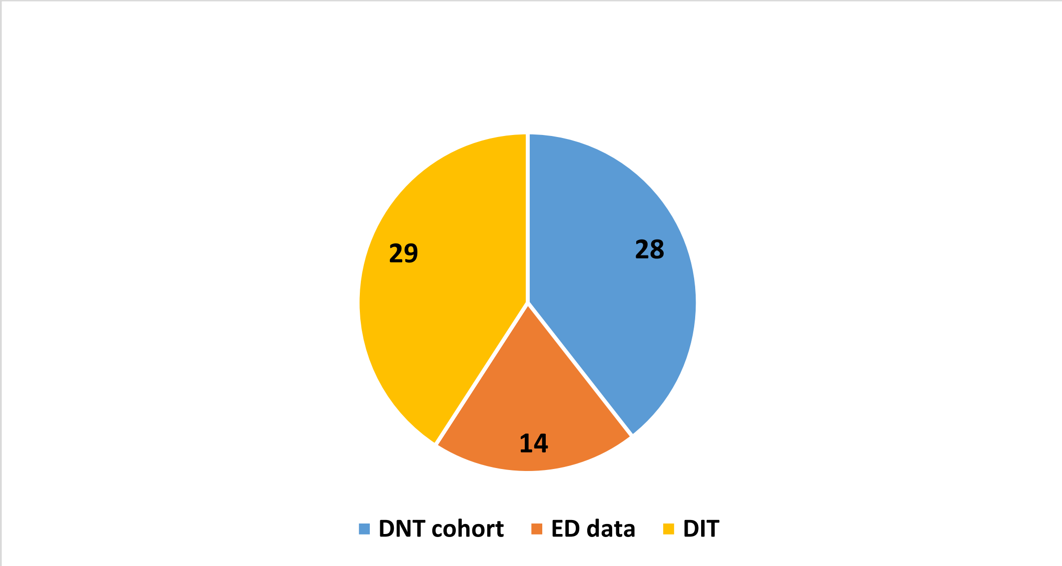


Figure 3. Distribution of the endpoint specific triggers from the open literature

Data obtained from ED databases on suspected ED MoA

Three databases (TEDX, SIN list and EU COM) were used in order to assess the potential endocrine disrupting properties of the investigated substances (Table 3).

Table 3. Statistical information on data obtained from ED databases

		Parent chemicals	Analogues	Constituents
TEDX		3	1	1
SIN list	Listed as ED	1	2	2
	Number of assessed substances that are similar to SIN substances	21	22	9
EU COM		0	0	0

Data obtained from Danish (Q)SAR database on suspected ED MoA

Positive (Q)SAR predictions on endocrine disrupting properties were found for 16 parent chemicals, 15 analogues and 9 constituents.

4.2. Outcome from MSC on the EOGRTS design

The outcome in the final decisions concerning the design of EOGRTS i.e. inclusion of cohorts F2, DNT, DIT after PfAs were submitted by MSCAs and discussed by MSC is reported below. The tables include both cases brought forward for discussion in the MSC by DK MSCA based on the search in this project and cases brought forward by other MSCAs in this time period. The cases are grouped by the type of the effect the PfAs were based on (see subsections 4.2.1 – 4.2.6.).

Until the date of drafting of this report (February 2017), 33 EOGRTS cases had been referred to MSC (meetings MSC 47 – MSC 52). The outcome of the EOGRTS design in the final decisions (after proposals were submitted and discussed by the MSC) had been presented for 19 substances. For the remaining 14 cases referred to MSC 52, the substances are listed but the outcome not included.

4.2.1. Endocrine Disrupting mechanism(s)/mode(s) of action

Table 4. Outcome of the DEV EOGRTS cases discussed at the MSC meetings (47-51), grouped by PfAs based on information on ED MoA.

MSC	Substance name	CAS number	EC number	PfA inclusion of cohorts	Indication of effect	Outcome in the final decision
48	2-dimethyl-amino-ethanol	108-01-0	203-542-8	F2	ED MoA (choline uptake), genotoxicity [ECHA, 2016b]	F2 not requested [ECHA, 2016b]
	Dioctyltin oxide	870-08-6	212-791-1	DNT	ED MoA [ECHA, 2016b]	DNT not requested [ECHA, 2016b]
	Methyl 2-naphthyl ether	93-04-9	202-213-6	DNT, DIT	ED MoA [ECHA, 2016b]	Decision to await for 90-day study results and thereafter reassess EOGRTS design [ECHA, 2016b]
	Reaction mass of 1-(1,2,3,4,5,6,7,8-octahydro-2,3,8,8-tetramethyl-2-naphthyl)ethan-1-one and […]	NS	915-730-3	F2	Potential ED MoA [ECHA, 2016b]	F2 not requested. Decision to await for 90-day study results and thereafter reassess EOGRTS design [ECHA, 2016b]
	Tert-butyl 2-ethylperoxyhexanoate	3006-82-4	221-110-7	DNT, DIT	ED MoA [ECHA, 2016b]	DNT not requested DIT not requested [ECHA, 2016b]
50	Turpentine oil	NS	932-349-8	DNT, DIT	ED MoA [ECHA, 2016c]	Decision to await for 90-day study results and thereafter reassess EOGRTS design [ECHA, 2016c]
	N-[3-(dimethylamino)propyl]methacrylamide	5205-93-6	226-002-3	F2	ED MoA [ECHA, 2016c]	No unanimous agreement reached→ case sent to EC [ECHA, 2016c]
	Diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide	75980-60-8	278-355-8	DNT, DIT	ED MoA [ECHA, 2016c]	DNT not requested DIT not requested

						[ECHA, 2016c]
	3,5-dimethylpyrazole	67-51-6	200-657-5	F2	ED MoA [ECHA, 2016c]	F2 requested [ECHA, 2016c]
	m-(2,3-epoxypropoxy)-N,N-bis(2,3-epoxypropyl)aniline	71604-74-5	275-662-9	DNT, DIT	ED MoA [ECHA, 2016c]	DNT not requested DIT not requested [ECHA, 2016c]
	Reaction mass of bis(2,3-epoxypropyl) terephthalate and tris(oxiranylmethyl) benzene-1,2,4-tricarboxylate	NS	940-592-6	DIT	ED MoA [ECHA, 2016c]	DIT not requested [ECHA, 2016c]
51	Ethanol, 2,2'-oxybis-, reaction products with ammonia, morpholine derivs, residues	68909-77-3	272-712-1	DNT, DIT	ED MoA [ECHA, 2016d]	Decision to await for 90-day study results and thereafter reassess EOGRTS design [ECHA, 2016c]

4.2.2. Acute neurotoxic effects

Table 5. Outcome of the DEV cases discussed at the MSC meetings (47-51), grouped by PfAs based on evidence for acute neurotoxic effects.

MSC	Substance name	CAS number	EC number	PfA inclusion of cohorts	Indication of effect	Outcome in the final decision
47	Benzaldehyde	100-52-7	202-860-4	DNT F2	Solvent toxicity; ROS formation, mutagenicity [ECHA, 2016f]	DNT not requested, F2 not requested [ECHA, 2016c]
48	2-ethylhexyl acrylate	103-11-7	203-080-7	DNT	Cholinesterase inhibition; *in vitro* data on neurotoxic effects on developing embryos and foetuses [ECHA, 2016b]	Registrant (REG) representative announced that new data will be available, based on RA through category approach. PfA withdrawn by the submitting MSC member [ECHA, 2016b]
51	Ethanol, 2,2'-oxybis-, reaction products with ammonia, morpholine derivs, residues	68909-77-3	272-712-1	DNT	Acute (and delayed) neurotoxic effects [ECHA, 2016d]	Decision to wait for 90-day study results and thereafter reassess EOGRTS design [ECHA, 2016d]

4.2.3. Sedative effects

Table 6. Outcome of the DEV cases discussed at the MSC meetings (47-51), grouped by PfAs based on evidence for sedative effects.

MSC	Substance name	CAS number	EC number	PfA inclusion of cohorts	Indication of effect	Outcome in the final decision
47	5-methylhexan-2-one	110-12-3	203-737-8	DNT	Sedative effect [ECHA, 2016e]	DNT not requested [ECHA, 2016e]

4.2.4. Other effects

Table 7. Outcome of the DEV cases discussed at the MSC meetings (47-51), grouped by PfAs based on evidence for other effects.

MSC	Substance name	CAS number	EC number	PfA inclusion of cohorts	Indication of effect	Outcome in the final decision
47	Benzaldehyde	100-52-7	202-860-4	Sequential testing for mutagenicity to gather data for triggering of F2	Potential Mutagen, Category 2 [ECHA, 2016e]	Not enough evidence to support PfA with sequential testing [ECHA, 2016e]
48	2-dimethyl-amino-ethanol	108-01-0	203-542-8	F2	Might cause genotoxicity through potential hypo/hypermethilation properties (also ED MoA) [ECHA, 2016b]	F2 not requested [ECHA, 2016b]
	Alcohols, C6-24 and C6-24-unsatd., distn. residues	102242-48-8	310-079-6	F2	Classified by the REG as Mutagen category 2, based on *in vitro* data [ECHA, 2016b]	F2 not requested. Decision to await for 90-day study results, and thereafter reassess EOGRTS design [ECHA, 2016b]
	Alcohols, C9-11-branched	68551-08-6	271-360-6	DNT, DIT	No detailed information provided in the minutes of the MSC meeting [ECHA, 2016b]	Decision to await for 90-day study results and thereafter reassess EOGRTS design [ECHA, 2016b]
	Dioctyltin oxide	870-08-6	212-791-1	DNT	Neurotoxicity through affecting the GABA, DA brain levels (also ED MoA) [ECHA, 2016b]	DNT not requested [ECHA, 2016b]
				DIT	Evidence of chronic immunotoxicity caused by dioctyltin chloride (structural analogue) [ECHA, 2016b]	DIT requested [ECHA, 2016b]
				F2	Proposal to remove F2 from the decision [ECHA, 2016b]	Proposal not approved, F2 still requested [ECHA, 2016b]
	Propyl acetate	109-60-4	203-686-1	90-day study requested	No detailed information provided in the minutes of the MSC meeting [ECHA, 2016b]	Decision to await for 90-day study results and thereafter reassess EOGRTS design [ECHA, 2016b]
	Reaction mass of 1-(1,2,3,4,5,6,7,8-octahydro-2,3,8,8-tetramethyl-2-naphthyl)ethan-1-one and […]	NS	915-730-3	Proposal to request the 90-day study and the EOGRTS in separate decisions.	The new information from the 90-day study might trigger the extension of cohorts [ECHA, 2016b]	Decision to await for 90-day study results and thereafter reassess EOGRTS design [ECHA, 2016b]
				DNT	Evidence of neurotoxic effects of acetyl ethyl tetramethyl tetralin, structural analogue of registered substance [ECHA, 2016b]	DNT requested [ECHA, 2016b]
	Tert-butyl 2-	3006-82-	221-110-	F2	Potential Mutagen	F2 not requested

	ethylperoxyhexanoate	4	7		Category 2 [ECHA, 2016b]	[ECHA, 2016b]
	Tetrahydrofurfuryl alcohol	97-99-4	202-625-6	Proposal to remove DNT and DIT cohorts.	The substance is already classified as reprotoxic (Repr 1B H360Df, Harmonised classification), and thus no further need for developmental toxicity testing [ECHA, 2016b]	Proposal not approved, DNT and DIT still required [ECHA, 2016b]
50	3,5-dimethylpyrazole	67-51-6	200-657-5	DNT	Non-impaired hearing combined with lack of startling response [ECHA, 2016c]	DNT requested [ECHA, 2016c]
	m-(2,3-epoxypropoxy)-N,N-bis(2,3-epoxypropyl)aniline	71604-74-5	275-662-9	DIT	Effects on blood-cell counts; substance classified as skin sensitizer (also ED MoA) [ECHA, 2016c]	DIT not requested [ECHA, 2016c]
	Reaction mass of bis(2,3-epoxypropyl) terephthalate and tris(oxiranylmethyl) benzene-1,2,4-tricarboxylate	NS	940-592-6	DIT	Substance classified as Skin Sensitizer 1 (also ED MoA) [ECHA, 2016c]	DIT not requested [ECHA, 2016c]

4.2.5. Results from MSC 52

Table 8. List of the DEV cases discussed at the 52nd MSC meeting [ECHA, 2016f]; the Minutes document of the 52nd MSC meeting was not publicly available at the time this publication was drafted.

MSC 52	Substance name	CAS number	EC number	PfA inclusion of cohorts*	Indication of effect*	Outcome in the final decision*
(February, 2017)	Reaction mass of dimethyl adipate and dimethyl glutarate and dimethyl succinate	NS	906-170-0	-	-	-
	(1-methyl-1,2-ethanediyl)bis[oxy(methyl-2,1-ethanediyl)] diacrylate	42978-66-5	256-032-2	-	-	-
	N-1,3-dimethylbutyl-N'-phenyl-p-phenylendiamine	793-24-8	212-344-0	-	-	-
	Cyclohexyl methacrylate	101-43-9	202-943-5	-	-	-
	Ionone, methyl	1335-46-2	215-635-0	-	-	-
	Dinitrogen tetraoxide	10544-72-6	234-126-4	-	-	-
	2,4,6-trichloro-1,3,5-triazine	108-77-0	203-614-9	-	-	-
	N-butylbenzene-sulphonamide	3622-84-2	222-823-6	-	-	-
	Oxirane, mono[(C12-14-alkyloxy)methyl] derivs.	68609-97-2	271-846-8	-	-	-
	1,3-dihydro-4(or 5)-methyl-2H-benzimidazole-2-thione, zinc salt	61617-00-3	262-872-0	-	-	-
	1,3-dihydro-4(or 5)-methyl-2H-benzimidazole-2-thione	53988-10-6	258-904-8	-	-	-
	2,2'-ethylenedioxydiethyl bis(2-ethylhexanoate)	94-28-0	202-319-2	-	-	-
	Ethanol, 2,2'-iminobis-, N-C12-18-alkyl derivs.	71786-60-2	276-014-8	-	-	-
	2,2-dimethylpropane-1,3-diyl cyclohex-4-ene-1,2-dicarboxylate	41026-17-9	255-180-5	-	-	-

***** The document entitled *Minutes of the 52nd meeting of the MSC*, regarding the decision made on the discussed EOGRTS cases, was made publicly available on ECHA's official website in April, 2017, and thus not available at the time this publication was drafted.

4.2.5. Cases with a change in the decision

Table 9. List of the DEV cases discussed at the MSC meetings, with changes in the decision adopted by ECHA (cases for which the extension of the EOGRTS design was requested, based on the submitted PfAs).

MSC	Substance name	CAS number	EC number	PfA inclusion of cohorts	Indication of effect	Outcome in the final decision
50	3,5-dimethylpyrazole	67-51-6	200-657-5	F2	ED MoA [ECHA, 2016c]	F2 requested [ECHA, 2016c]
				DNT	Impaired startling response [ECHA, 2016c]	DNT requested [ECHA, 2016c]

5. Discussion and conclusion

This project contributes to the implementation of REACH regulatory requirements for the EOGRTS, at an early stage, when there is little experience in interpretation of data for triggering of the F2, DNT and/or DIT cohorts.

Information gathered from the open scientific literature may, for some substances, indicate potential effects on the developmental neuro- and immune systems, and be used to trigger the test design allowing such specific investigations under REACH. Our analysis showed that the open scientific literature search resulted in retrieving of potentially relevant information for triggering of the F2, DNT and/or DIT cohorts for 48 out of 121 assessed parent chemicals (39 % of analysed cases). Most of the findings were related to the parent substances, a significant number was also retrieved by searching analogues (26/82) and constituents (10/46) for the multi-constituent or UVCB substances. The retrieved potentially relevant information was linked to DNT (28 substances), DIT (29 substances) and ED MoA (14 substances), the endocrine disrupting data potentially being used for triggering of all three EOGRTS cohorts. These results indicate that there has been some interest of the scientific community to investigate effects of substances produced or imported at high tonnage in the EU. However, the awareness about scientific questions that are of regulatory interest and substances that are under regulatory scrutiny could be increased by improved communication between regulatory authorities and scientists in the relevant fields. ECHA is continuously improving the regulatory transparency via its dissemination website and could further consider those elements.

Until the date of the drafting of this report (February 2017), 33 substances have been discussed from an EOGRTS perspective at the 47-52 MSC meetings. This early experience from the MSC discussions on the design of EOGRTS shows the lack of immediate success in using the scientific publications from the open literature in triggering of specific investigations for DNT, DIT or F2 in EOGRTS. For those still very few discussed and analysed cases (19 substances), PfAs submitted for one substance led to triggering of both DNT and F2. The systematic evaluation of the reasons for the lack of acceptance by ECHA and several member states for the PfAs and the triggers therein was not in the scope of this project (and may be difficult due to the limited reporting).

The MSC discussions identified several general scientific questions that could be addressed by the scientific community and/or regulatory authorities. Those include e.g.:

1. Are narcotic effects in the acute or repeated dose studies with adult animals a basis for concern for developmental neurotoxicity of the substance, and what are the most sensitive DNT endpoints?

2. What are the most sensitive DNT endpoints to test developmental neurotoxicity caused by ED substances acting through interference with the sex hormone system?

3. Related to DNT, could the cognitive tests be included without jeopardising validity of EOGRTS?

References

ECHA, 2015. Guidance on Information Requirements and Chemical Safety Assessment, Chapter R.7a: Endpoint specific guidance, Version 4.1, October 2015.

ECHA, 2016a. How ECHA identifies the design for the extended one-generation reproductive toxicity study (EOGRTS) under dossier evaluation, September 2016.

ECHA, 2016b. Minutes of the 48th Meeting of the Member State Committee (MSC-48), June 2016; retrieved from https://echa.europa.eu/documents/10162/22837890/msc-48_meeting_minutes_en.pdf/314824ca-fd22-4d96-ae8d-3ba2050341f7 , accessed on 13.02.2017.

ECHA, 2016c. Minutes of the 50th Meeting of the Member State Committee (MSC-50), October 2016; retrieved from https://echa.europa.eu/documents/10162/22837890/msc-50_minutes_en.pdf/c6a264b7-b23e-a94a-2fb8-1e054455a76b , accessed on 13.02.2017.

ECHA, 2016d. Minutes of the 51st Meeting of the Member State Committee (MSC-51), December 2016; retrieved from https://echa.europa.eu/documents/10162/22837890/msc-51_minutes_en.pdf/9a506e8a-5e7d-80ed-0e6f-6ef8aa65a0de, accessed on 13.02.2017.

ECHA, 2016e. Minutes of the 47th Meeting of the Member State Committee (MSC-47), April2016; retrieved from https://echa.europa.eu/documents/10162/22837890/msc-47_meeting_minutes_en.pdf/5c0a51cf-181b-4fa5-8818-a75becf26c8c, accessed on 10.02.2017.

ECHA, 2016f. Draft Agenda of the 52nd meeting of the Member State Committee, February 2017; retrieved from https://echa.europa.eu/documents/10162/22837890/msc-52_agenda_en.pdf/5d7cce3e-4692-0431-5292-abccad361fe2 , accessed on 17.02.2017.

ECHA, 2017. Regulations: Evaluation, retrieved from https://echa.europa.eu/regulations/reach/evaluation/evaluation-procedure, accessed on 3.02.2017.

Appendix I: Relevant data obtained through the conducted open literature search

This information could not be made public at the time this report was published, due to a potential breach of confidentiality.

Appendix II: All assessed chemicals

This information could not be made public at the time this report was published, due to a potential breach of confidentiality.

Appendix IV: Identified metabolites of the assessed substances, using the OECD QSAR Toolbox program

This information could not be made public at the time this report was published, due to a potential breach of confidentiality.

Appendix III: Type of metabolites, according to the OECD QSAR Toolbox program, Version 3.4, 2016

DOCUMENTED	
Type of metabolism	The OECD QSAR Toolbox uses a database that contains information about:
Observed mammalian	"Metabolic pathways documented for chemicals with studies in different mammals."
Observed microbial	"Degradation pathways used by microorganism to obtain carbon and energy from substances."
Observed rat in vivo	"Documented metabolic pathways, extracted from scientific literature and associated with in vivo biotransformation of xenobiotic chemicals in rodents."
Observed rat liver S9	"Documented metabolic pathways for chemicals observed with the use of in vitro experimental systems such as rodent (mostly rat) liver microsomes and S9 fraction."
SIMULATED	
Type of simulator	The OECD QSAR Toolbox uses a database that contains information about:
Autooxidation, Autooxidation (alkaline medium)	"Autoxidation (AU) is a spontaneous, air-induced oxidation of organic molecules. It is a free- radical chain reaction of a chemical with molecular oxygen, resulting in the formation of oxidation products. Among the latter, organic hydroperoxides are regarded as the most important with respect to eliciting adverse effects such as contact allergy. An AU model was therefore developed to simulate the observed AU pathways. The AU simulator consists of a set of molecular transformations, extracted from the observed AU pathways."
Dissociation	"Dissociation simulation module."
Hydrolysis (acidic medium), Hydrolysis (basic medium)	"Hydrolysis simulator (acidic / basic) predicts hydrolysis products of discrete organic chemicals under the following experimental conditions: acidic pH, ambient or room temperature and atmospheric pressure. The simulator was developed based on data collected from various sources, including articles and public web sites."
Hydrolysis (neutral medium)	"Hydrolysis simulator is an abiotic model and predicts the hydrolysis products of chemicals and their quantities at 28 day under neutral or nearly neutral pH. The model predicts hydrolysis products of discrete organic chemicals under the following experimental conditions: neutral or nearly neutral pH, ambient or room temperature and atmospheric pressure. The simulator was developed based on data collected from various sources, including articles and public web sites."
In vivo rat metabolism	"The current in vivo rat liver metabolic simulator (transformation table) represents electronically designed set of 609 structurally generalized, hierarchically arranged abiotic and enzymatic transformation reactions, which are characteristic for the metabolism for in vivo experimental systems such as rodent (mostly rat). The principal applicability of this simulator is associated with the reproduction as well as the prediction of the metabolic activation reactions and pathways of xenobiotic chemicals, which may elicit in vitro genotoxicity effects such as bacterial mutagenicity and chromosomal aberrations. Each transformation in simulator consists of source and product structural fragments, and inhibiting "masks". A probability of occurrence is ascribed to each principal transformation, which determines its hierarchy in the transformation list. A training set of xenobiotic chemicals of a wide structural diversity, with experimentally observed metabolic reactions and pathways has been built, using published data on their liver metabolism. The data on their metabolism are collected mostly from research publications in the field from selected scientific journals, monographs and websites, and are associated with the commonly observed in vivo liver metabolic reactions of chemicals with different structures."
Microbial metabolism	"Multiple pathway catabolism is simulated using the abiotic and enzyme-mediated reactions via the hierarchically ordered principal molecular transformations extracted from documented metabolic pathway database. The hierarchy of the transformations is used to control the propagation of the catabolic maps of the chemicals. The simulation starts with the search for match between the parent molecule and the

	source fragment associated with the transformation having the highest hierarchy. If the match is not found search is performed with the next transformation, etc. When the match is identified, the transformation products are generated. The procedure is repeated for the newly-formed products. Predictability (probability that the metabolite is observed, given that the metabolite is predicted) evaluated on the bases of documented catabolism for 200 chemicals stored in the database of <<Observed microbial catabolism>> is 83%."
Rat liver S9 metabolism	"The current in vitro rat liver metabolic simulator (transformation table) represents electronically designed set of 509 structurally generalized, hierarchically arranged biotransformation reactions, which are characteristic for the metabolism for in vitro experimental systems such as rodent (mostly rat) liver microsomes and S9 fraction. The principal applicability of this simulator is associated with the reproduction as well as the prediction of the metabolic activation reactions and pathways of xenobiotic chemicals, which may elicit in vitro genotoxicity effects such as bacterial mutagenicity and chromosomal aberrations. A training set of xenobiotic chemicals of a wide structural diversity, with experimentally observed metabolic reactions and pathways has been built, using published data on their metabolism in rodent liver microsomes and S9 fraction."
Skin metabolism	"Skin metabolism simulator mimics the metabolism of chemicals in the skin compartment. Given the lack of reported skin metabolism data and the widespread hypotheses is that skin enzymes can metabolize absorbed xenobiotics via reactions analogous to those determined in liver, the simulator was developed as a simplified mammalian liver metabolism simulator. The skin metabolism simulator contains a list of hierarchically ordered principal transformations, which can be divided into two main types – rate-determining and non-rate-determining. The rate-determining transformations are Phase I and Phase II, such as C-hydroxylation, ester hydrolysis, oxidation, glutathione conjugation, glucuronidation, sulfonation. The non-rate-determining transformations include molecular transformations of highly reactive intermediates. The simulator starts by matching the parent molecule with the reaction fragments associated with the transformation having the highest probability of occurrence. This produces a set of first level metabolites. Each of these derived metabolites is then submitted to the same list of hierarchically ordered transformations, to produce a second level of metabolites. "