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Use of QSAR to identify potential CMR substance of relevance under the REACH regulation

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Use of QSAR to identify potential CMR substances of relevance under the REACH regulation

Environmental Project No. 1503, 2013

Content

Preface

This documentation report describes a project carried out by QSAR researchers from the National Food Institute, Technical University of Denmark (DTU). In the project QSAR models have been applied to screen REACH substances for potential CMR properties. The substances screened were either not registered by the deadline in 2010 or have been registered but not classified for CMR properties.

The project participants from DTU were:
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The project steering group members were besides the participants from the DTU also Henrik Tyle and Magnus Løfstedt, both from the Danish Environmental Protection Agency (EPA).

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The report reflects the author's views and opinions but not necessarily views and opinions of the Danish EPA.

Summary and Conclusion

In the European chemicals legislation, REACH /1/, the first deadline for registration was the 1st December 2010. At this deadline, pre-registration substances (PRS) living up to at least one of a number of criteria in REACH articles should be registered. The criteria in REACH art. 12(1) specify that substances classified as carcinogenic, mutagenic or toxic to reproduction (CMR) in category 1 or 2 according to Directive 67/548/EEC /2/, and manufactured in or imported into the EU in quantities of 1 tonne or more per year per manufacturer or per importer, should be registered. Of the 143,835 PRS, 5,705 substances were according to the European Chemicals Agency, ECHA, registered per 15th June 2012 and the published part of the list was available as a downloadable file on ECHA's homepage /3/.

However, earlier studies have shown that a very large fraction of the high tonnage industrial substances on the EU market have few or no experimental test data /4/. As a result, substances presently used in the EU with CMR properties, may not have been recognized, and consequently not self-classified for CMR and registered under REACH. Furthermore, registered substances with unrecognized CMR properties may similarly not have been suggested classified for CMR effects.

Because experimental determination of toxicity requires resources both in terms of cost and time, reliable *in silico* alternatives such as quantitative structure-activity relationship (QSAR) models are becoming important tools for rapid and cost-effective predictions of toxic effects. In this project QSAR models were used to screen REACH substances for potential CMR properties. The individual models applied are according to validation results able to predict the individual endpoints with accuracies (overall concordances) of approximately 70-85 percent, with most of the models having considerably higher specificity than sensitivity.

Model predictions from different models for relevant tests were combined to reach overall CMR calls. The QSAR model prediction algorithms used for C or M calls require positive predictions from more than one of the included QSAR models. This may theoretically reduce "noise" (i.e. erratic/erroneous occurrence of data which are inconsistent when evaluated across related test endpoints or test systems) and increase accuracy in the overall call. For C the screening was limited to genotoxic carcinogens and for R only certain types of mechanisms causing malformation or fetal mortality were included. This means that non-genotoxic carcinogens or reproductive toxicants having other types of effects were not covered in the screening.

QSARs were applied to screen for CMR effects for:
1. Substances registered in 2012 that are not self-classified by industry as C and/or M and/or R.
2. Pre-registered substances which were not registered in 2012, possibly due to unrecognized CMR properties based on lack of test data.

QSAR-based CMR screenings were applied for REACH substances where structure information was available; 1,066 substances registered under REACH by 2012 and 67,656 PRS not registered were screened. For the non-registered substances information from the Nordic substance register database (SPIN) was used to identify substances for which human exposure is likely to be significant.

Within the constraints of the project it was chosen to provide a more thorough analysis on a few manually selected substances for which supporting information was also taken into consideration.

This included a quick check of possible readily available existing experimental CMR information on the eChemPortal website. Furthermore, the in-house Danish QSAR prediction database was taken into account for a more holistic evaluation of the individual substances. The database contains predictions for many endpoints covering physical-chemical properties, bioavailability, CYP P450 metabolism, eco-toxicity and human health endpoints (mainly effects in rodents measured by standardized laboratory tests.

The screening resulted in the following numbers of REACH substances predicted by QSAR to potentially have CMR properties:
1. Non-registered substances in 2012; 18,266
2. Non-registered substances in 2012 with likely significant human exposure; 695
3. Non-registered substances in 2012, with exposure information and manually selected; 26
4. Registered substances (of which some may be classified for CMR); 212
5. Registered substances not classified for CMR (by a harmonized classification or by a self-classification of the REACH registrant), manually selected; 5

The resulting lists of substances with predicted CMR properties were generated with the aim that the Danish EPA can use them as a tool for prioritizing substances for further work.

It is important to note that the substances mentioned in this report have not proven to meet the criteria for carcinogenicity, mutagenicity or reproductive toxicity. Nor has it been proven that their use constitute a risk to consumers or workers. Further work needs to be carried out including expert assessment of all available relevant information for each substance before a conclusion on their CMR properties can be made.

For the registered substances (point 4) a further expert evaluation can be undertaken in relation to the prioritization of substances for the CoRAP list (the EU list of registered substances, which are examined to clarify suspicion about effects of concern). This prioritization takes place once a year and is done by the European Chemicals Agency (ECHA) in cooperation with the member states.

For the non-registered substances with likely significant human exposure (point 2), the QSAR results from this exercise can be applied to flag to Industry, that there may be a need to obtain more knowledge in order to document a safe use (depending on which available documentation already exists).

Sammenfatning

Den første frist for registrering under den europæiske kemikalielovgivning REACH /1/ var den 1. december 2010. Denne dato skulle alle såkaldte præ-registreringsstoffer (PRS) som lever op til visse kriterier registreres. Ifølge REACH art. 12(1) skulle blandt andet stoffer som er klassificeret som kræftfremkaldende, mutagene eller toksiske for reproduktionen (CMR) i kategori 1 eller 2 ifølge Direktiv 67/548/EEC/2, og som produceres eller importeres i EU i mængder på 1 ton eller mere per år per producent eller importør registreres. Af de 143.835 PRS var 5.705 kemiske stoffer per 15. juni 2012 blevet registreret ifølge det Europæiske Kemikalieagentur ECHA, og den publicerede del af listen var tilgængelig på ECHA's hjemmeside /3/.

Tidligere studier har imidlertid vist, at en meget stor andel af højtonnage industrikemialierne på markedet i EU har mangelfulde eller ingen eksperimentelle data /4/. Konsekvensen af dette er, at nogle CMR stoffer anvendt i EU muligvis ikke er blevet erkendt som havende disse egenskaber og dermed ikke er blevet selv-klassificeret for CMR og registreret under REACH. Endvidere er registrerede høj-tonnage stoffer med ikke erkendte CMR egenskaber højst sandsynligt ikke blevet foreslået klassificeret for CMR effekter.

Eksperimentel undersøgelse af toksicitet kræver ressourcer både i form af tid og penge, og derfor er pålidelige *in silico* alternativer som for eksempel QSAR (quantitative structure-activity relationship) modeller blevet vigtige værktøjer i forbindelse med hurtige og omkostningseffektive forudsigelser af toksiske effekter. I dette projekt blev QSAR modeller brugt til at screene REACH stoffer for potentielle CMR egenskaber. Modellerne anvendt i projektet for de individuelle effekter har ifølge valideringsresultater overordnede nøjagtigheder på omkring 70-85%, hvor de fleste af modellerne har en betydeligt højere specificitet end sensitivitet.

Modelforudsigelser fra forskellige modeller for relevante tests blev kombineret i QSAR model algoritmer der mundede ud i overordnede CMR meldinger. Algoritmerne for C og M kræver positive forudsigelser fra mere end én af de inkluderede QSAR modeller. Dette kan teoretisk reducere "støj" (tilfældig/fejlagtig forekomst af data som er inkonsistente når der evalueres på tværs af relaterede effekter eller test systemer) og forhøje nøjagtigheden i den overordnede melding. For C var screeningen begrænset til genotoksiske kræftfremkaldende stoffer og for R var kun visse typer af mekanismer der giver misdannelser eller fosterdød inkluderet. Dvs. at ikke-genotoksiske kræftfremkaldende stoffer og reproduktions-toksiske stoffer med andre typer af effekter ikke var inkluderet i screeningen.

QSAR modellerne blev anvendt til screening af CMR effekter for:
1. Stoffer registreret per 2012, men ikke selv-klassificeret af industrien som C og/eller M og/eller R.
2. Præ-registrerede stoffer som ikke var registrerede i 2012 muligvis på grund af ikke erkendte CMR egenskaber forårsaget af mangel på test data.

QSAR baserede CMR screeninger blev anvendt på REACH stoffer for hvilke struktur information var tilgængelig; 1.066 stoffer registreret under REACH per 2012 og 67.656 PRS blev inkluderet i screeningen. For de ikke registrerede stoffer blev information fra den nordiske stofregister-database (SPIN) desuden anvendt til at identificere stoffer med potentiel human eksponering.

I det omfang projektet tillod det, blev et begrænset antal manuelt udvalgte stoffer underlagt en mere grundig analyse, hvor også supplerende information blev inddraget. Dette inkluderede en

hurtig gennemgang af mulig tilgængelig eksisterende eksperimentel CMR information på eChemPortal hjemmesiden. Desuden blev den danske in-house QSAR database, som indeholder forudsigelser for mange effekter såsom fysisk-kemiske egenskaber, biotilgængelighed, CYP P450 metabolisme, øko-toksicitet og humane sundhedseffekter (primært effekter målt i gnavere og udført efter standardiserede dyreforsøgs-protokoller), anvendt for at give en mere holistisk evaluering af de individuelle stoffer.

Screeningen resulterede i følgende antal af REACH stoffer med potentielle CMR egenskaber forudsagt ved hjælp af QSAR:

1. Ikke registrerede stoffer i 2012; 18,266
2. Ikke registrerede stoffer i 2012 med signifikant human eksponering; 695
3. Ikke registrerede stoffer i 2012 med eksponeringsinformation og manuelt udvalgt; 26
4. Registrerede stoffer (hvoraf nogle muligvis er klassificeret for CMR); 212
5. Registrerede stoffer ikke klassificerede for CMR (ved harmoniserede klassificeringer eller selvklassificeringer af REACH registranten), manuelt udvalgt; 5

De resulterende lister over stoffer med forudsagte potentielle CMR egenskaber blev genereret med det formål at Miljøstyrelsen kan bruge dem fremadrettet som et redskab til at prioritere indsatsen i forhold til kemiske stoffer.

Det er vigtigt at understrege, at det ikke er bevist at stofferne på listerne har kræftfremkaldende, mutagene eller reproduktions-toksiske effekter. Ligeledes er det heller ikke bevist at brugen af dem udgør en risiko for arbejdstagere eller forbrugere. Yderligere arbejde, der omfatter ekspertvurderinger af al relevant tilgængelig information for de enkelte stoffer, skal udføres før en konklusion omkring deres potentielle CMR egenskaber kan drages.

For de registrerede stoffer (punkt 4) kan en yderligere ekspertvurdering foretages i forbindelse med prioritering af stoffer til CoRAP listen (EU liste over registrerede stoffer, der skal undersøges nærmere med henblik på at afklare mistænkte bekymrende effekter). Denne prioritering finder sted en gang om året og foretages af det Europæiske Kemikalieagentur (ECHA) i samarbejde med medlemslandene.

For de ikke-registrerede stoffer med eksponering (punkt 2) kan QSAR resultaterne fra denne undersøgelse bruges som et signal til Industrien om, at der kan være behov for fremskaffelse af mere viden for at dokumentere sikker brug (afhængigt af, hvilken anden dokumentation der allerede foreligger).

Introduction

Background

According to the chemicals legislation in the EU, REACH, all CMR substances (Carcinogenic, Mutagenic, or toxic to Reproduction) produced or imported in more than 1 tonne per year should be registered by 1 December 2010. The same applies according to REACH art. 23 to vPvB/PBT- (very Persistent very Bioaccumulating / Persistent, Bioaccumulating and Toxic) substances and substances classified as very toxic to aquatic organisms which may cause long-term adverse effects in the aquatic environment (R50/53) above 100 tonnes per year, and finally to all high production volume substances (>1000 tonnes per year per EU producer/importer) regardless of their hazardous properties.

However, earlier studies /4/ of the previous list of existing industrial substances in the EU, the so-called EINECS list, have shown that for the majority of the EINECS high production volume substances there were insufficient or no test data about their CMR properties. For the less-than-high tonnage substances there is likely to be even less test data about their CMR properties.

With the new REACH pre-registration list (PRS), the number of industrial substances in the EU has grown from 100,206 EINECS substances to potentially 143,835 substances. It is to be expected that for this new list, which includes the full EINECS list, there may be many substances with insufficient or no experimental results on CMR properties.

Due to the lack of test data on CMR properties, unrecognized CMR substances presently used in the EU in tonnages above 1 tonne and less than 1000 tonnes per year per importer/producer may with high probability not have been registered under REACH as foreseen by the legislation.

The Advisory self-classifications list /5-8/based on QSARs and published by the Danish Environmental Protection Agency (2001, 2009, 2010) indicates that there may be a substantial number of the substances on the EINECS list, and consequently on the PRS list, with non-recognized CMR properties.

Aim

The aim of the project was to generate working lists of relevant substances with potential CMR properties, which can be used by the Danish EPA as a tool for prioritizing substances for further work. For the generation of the working lists QSARs were applied to screen for potential CMR properties. See annex 1 for a general introduction to QSARs and annex 4 for the QSAR models used. Where structural information for discrete organic substances was available, QSAR models were applied to screen for CMR properties for REACH substances, which were either not registered or which were registered but not classified for CMR properties. Furthermore, information from the Nordic substance register database (SPIN) was used to identify substances where human exposure is likely to be significant.

QSAR screening and results

An overview of the steps and resulting numbers of selected substances in the project is given in Figure 1.

Not registered substances		Registered substances
Start lists		
143,835*		5,306**
Step 1 Selection of substances for QSAR screening		
69,438		1,545
Step 2 Removal of substances with EU classification		
67,656		1,066
	Step 3 CMR class. filter	
		969
Step 4 Selection of CMR predicted substances		
18,266		212
Step 5 SPIN filter		
695		
Step 6 Manual selection of cases for illustration		
26		5
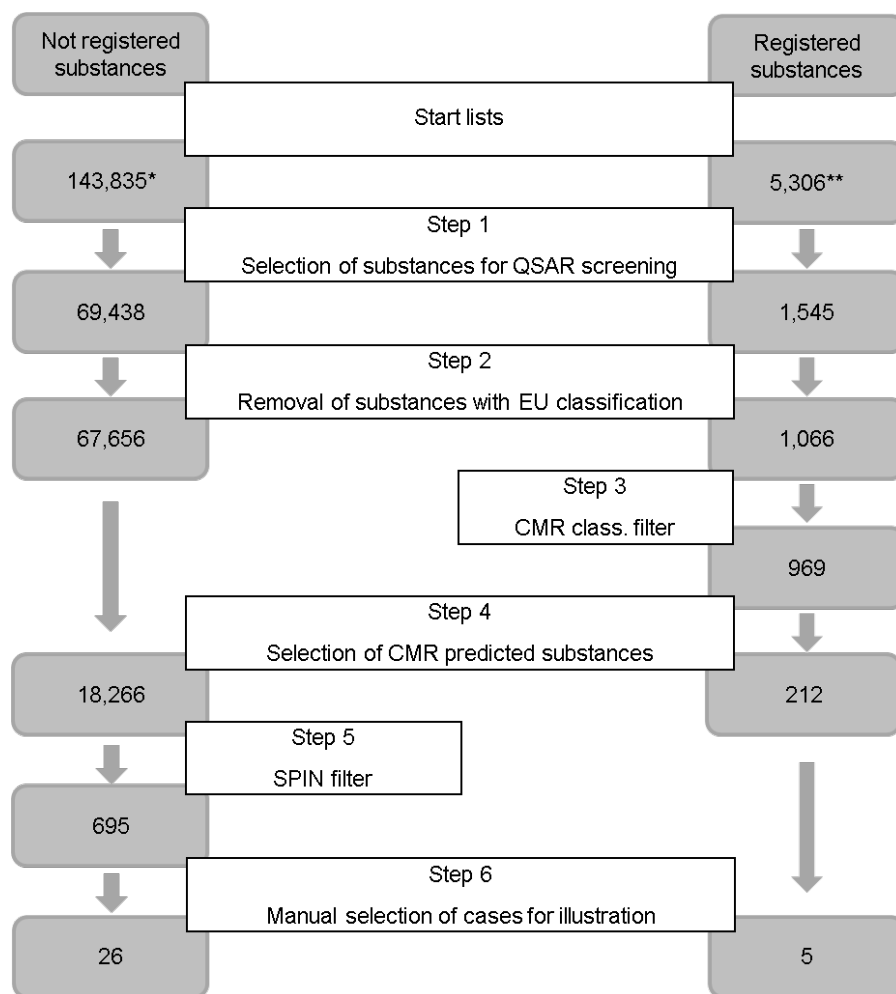
* Total number of pre-registered substances. Of these, the registered part (4,303 substances according to an EC number comparison) is treated separately in the right side of Figure 1

** Publishable registered chemicals from ECHA homepage, downloadable file as of 15th June 2012

Figure 1 Overview of the project flow

Initial lists

The initial lists for the screening were the ECHA list of registered substances as of 15th June 2012 with 5,705 registered substances of which 5,306 were publishable /3/, and the ECHA pre-registration list with 143,835 substances, which were pre-registered (PRS) between 1 June and 1 December 2008 /10/.

Selection of structures for QSAR screening (step 1 in Figure 1)

To make the QSAR screening as comprehensive as possible it should be performed on as many as possible of the substances on the two initial lists. A prerequisite for making predictions with the QSAR software is that structural information in the format of e.g. SMILES or sdf representation is available, and that it is discrete organic substances.

As structural information was not required as part of the pre-registration and as no formal structure set on the PRS from ECHA exists, other sources for the structural information were searched. The Computational Toxicology Group within the EC Joint Research Centre (JRC) has generated structure information for as many as possible of the PRS by using the ACDLabs Name-To-Structure software and validated them with a random sample /9/. In total 80,413 structures were generated, including discrete organics, inorganics /organometallics etc.

The remaining around 60,000 PRS not included in the JRC list may be e.g. discrete organic structures where structure information could not be retrieved or UVCB's (substance of Unknown or Variable composition, Complex reaction products or Biological materials, e.g. extracts from plants, which are only suitable for QSAR analysis if the individual components are identified).

Of the 80,413 structures on the JRC list, 70,983 substances could be applied in the QSAR screening software used in this project. Of these, 1,545 were on ECHA's list of "Publishable Substances Registered as of 15-Jun-2012" with a total of 5,306 published entries, and the remaining 69,438 were PRS, which were not registered. See Annex 2 for a description of the preparation of the JRC structure set for this project.

Removal of substances with EU harmonized classification (step 2 in figure 1)
Substances with EU harmonized classifications on Annex VI to Regulation (EC) No 1272/2008 /11/ including ATP1, and Annex I to Directive 67/648/EEC /12/ including ATP 31 were removed by applying a CAS number comparison. This comparison included, where information was available, also group expansions for group entries. However, as there is no official exhaustive CAS list of all substances covered by the group entries in Annex VI, and because a substance may have more than one CAS number, a few substances with EU harmonized classifications may not have been removed in this step.

A total of 1,066 REACH registered substances and 67,656 PRS not registered remained for processing.

CMR classification filter; removal of substances with industry CMR self-classifications (step 3 in figure 1)
REACH registration dossiers for the 1,066 substances were downloaded from the ECHA website (for some substances multiple dossiers), and the CMR classification information was extracted from these. The classifications in the dossiers may be harmonized EU classifications or they may be self-classifications. Step 2 filtered out all the EU harmonized classified substances listed with CAS numbers but there may be chemicals in the 1,066 list covered by harmonized classification group entries. From the 1,066 substances there were 97 substances having at least one CMR R-phrase (R40, R45, R46, R49, R60, R61, R62, R63 or R68). (It can be mentioned in parentheses that of the 97 substances having a CMR classification in the registration dossier, there were 48 which were predicted to be C, M and/or R by the QSAR screen applied in step 4).

A total of 969 REACH registered substances remained for processing.

As an additional exercise, all PRS having one or more self-classifications for C, M or R notified from one or more manufacturers/importers were downloaded from the ECHA C&L Inventory database /13/. Out of the 70,983 JRC substances which could be applied in the QSAR screening 2,239 (3.2%) had one or more self-classifications for C, M or R in ECHAs database. Of the substances predicted

in step 4 to have CMR properties (for not registered substances 18,266 substances and for registered substances 212 substances) there were 753 (4.1%) not registered and 31 (14.6%) registered substances with one or more Industry notified self-classifications for C, M or R.

Selection of CMR predicted substances (step 4 in figure 1)

The resulting lists of substances were submitted to MultiCASE (version 2.3.0.37) for generation of QSAR predictions for all relevant endpoints. The QSAR predictions from the individual models were integrated by decision algorithms for C-, M- and R- classification. These algorithms were also used in the development of the Danish Advisory self-classification list /7,8/.

The following commercial or in-house DTU QSAR models have been applied in the screening:

- Reverse mutation test, Ames *in vitro*
- Chromosome aberration in CHO *in vitro*
- Chromosome aberration in CHL *in vitro*
- Mouse lymphoma cell gene mutation test *in vitro*
- Sex-Linked Recessive Lethal test in Drosophila melanogaster *in vivo* (indicator test for structural chromosome aberration)
- Comet assay in mouse *in vivo* (indicator test for DNA damage/mutation)
- Sister chromatid exchange assay in mouse bone marrow *in vivo* (indicator test for structural chromosome aberration)
- Rodent dominant lethal test *in vivo* (indicator test for structural chromosome aberration)
- Mouse mammalian bone marrow erythrocyte micronucleus test *in vivo* (test for structural chromosome aberration)
- FDA Cancer models male/female Rat and male/female Mouse and RCA methodology
- Teratogenic potential in humans *in vivo* (based on epidemiological, clinical and animal data for drugs)

The models applied are according to validation results able to predict the individual endpoints with overall concordances of approximately 70-85 percent. See Annex 3 for an introduction to the MultiCASE software, and Annex 4 for technical information on the individual models.

Predictions from all the applied models for the full lists of substances were integrated into the Oasis DatabaseManager program, in which the screenings were performed according to the algorithms described below.

QSAR screening algorithm for genotoxic carcinogenicity

QSAR models for four carcinogenicity *in vivo* endpoints and three genotoxicity *in vitro* endpoints were included in the screening performed according to the scheme illustrated in Figure 2.

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┌──────────────────────────────────────────────────────────────┐
│   ┌────────────────────────────────────────────┐             │
│   │  Assessment of carcinogenicity             │             │
│   │                                            │             │
│   │  Predictions from models for in vivo       │             │
│   │                                            │             │
│   │   •  Carcinogenicity, male rat             │             │
│   │   •  Carcinogenicity, female rat           │             │
│   │   •  Carcinogenicity, male mouse           │             │
│   │   •  Carcinogenicity, female mouse         │             │
│   └────────────────────────────────────────────┘             │
│                      │                                        │
│                      │   Positive prediction according to     │
│                      │   the FDA RCA method, or positive      │
│                      │   experimental test                    │
│                      ▼                                        │
│   ┌────────────────────────────────────────────┐             │
│   │  Genotoxicity screening                    │             │
│   │                                            │             │
│   │  Predictions from models for in vitro      │             │
│   │                                            │             │
│   │   •  Reverse mutation test (Ames)          │             │
│   │   •  Chromosome aberration (CHO/CHL)       │             │
│   │   •  Mouse lymphoma                        │             │
│   └────────────────────────────────────────────┘             │
│                      │   Positive prediction or               │
│                      │   positive experimental test           │
│                      │   in at least one model                │
│                      ▼                                        │
│         ┌──────────────────────────────────┐                 │
│         │      Genotoxic carcinogen        │                 │
│         └──────────────────────────────────┘                 │
└──────────────────────────────────────────────────────────────┘
```
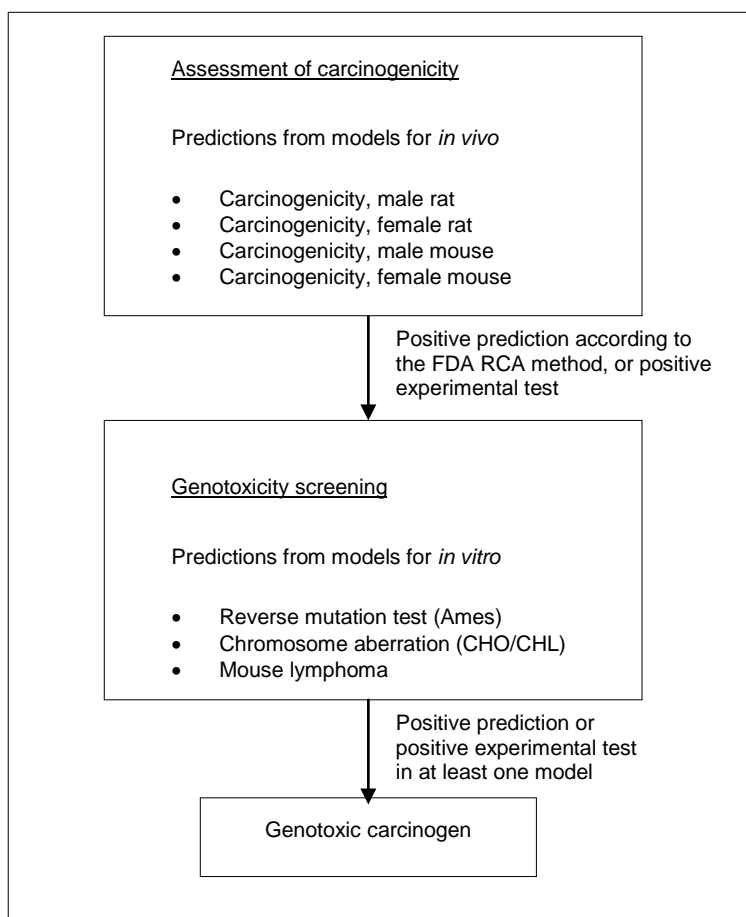
Figure 2 QSAR screening scheme for genotoxic carcinogenicity

The criterion for a positive cancer prediction is that there is a positive prediction according to the RCA methodology (previously denoted ICSAS) /14/, corresponding to two or more positive carcinogenicity predictions (within the applicability domain, AD, defined in the validation) with certain additional consistency requirements, and accepting only predictions for substances without significant deactivating fragments. If, for an evaluated substance, one or more positive experimental tests was present as part of the training sets for the models for any cancer endpoint, this took precedence over model predictions.

The genotoxicity criterion is a positive prediction (within the AD defined in the validation) from one or more of the models for the following *in vitro* genotoxicity endpoints; Reverse mutation test (Ames), chromosomal aberrations (CHO/CHL), or mutations in mouse lymphoma.

It is noted that the QSAR prediction algorithm used for the carcinogenicity call was restricted to genotoxic carcinogenicity. Hence this QSAR based carcinogenicity call did not address non-genotoxic carcinogens.

QSAR screening algorithm for mutagenicity
Five models predicting genotoxicity *in vivo* endpoints are included in the screening which is performed according to the scheme illustrated in Figure 3.
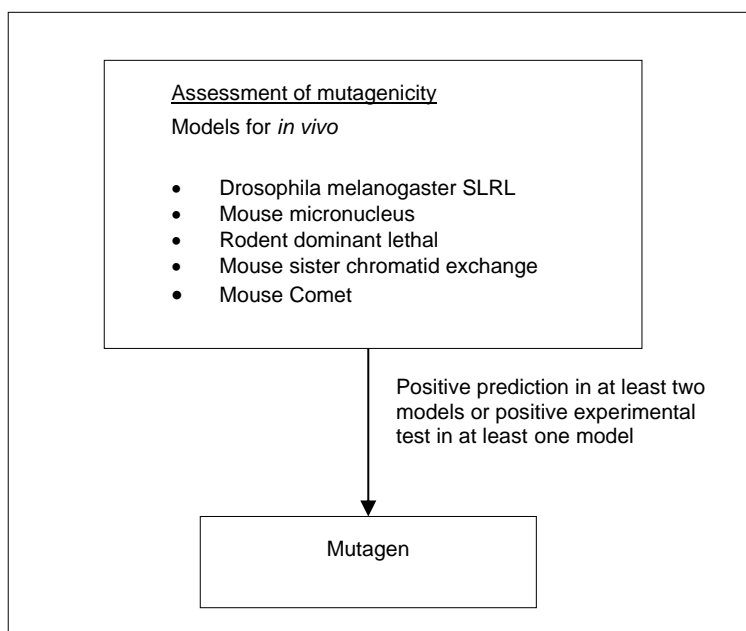
```
┌─────────────────────────────────────────────────────────┐
│   ┌─────────────────────────────────────────────┐       │
│   │  Assessment of mutagenicity                  │       │
│   │  Models for in vivo                          │       │
│   │                                              │       │
│   │    •  Drosophila melanogaster SLRL           │       │
│   │    •  Mouse micronucleus                     │       │
│   │    •  Rodent dominant lethal                 │       │
│   │    •  Mouse sister chromatid exchange        │       │
│   │    •  Mouse Comet                            │       │
│   └─────────────────────────────────────────────┘       │
│                    │                                     │
│                    │  Positive prediction in at least two │
│                    │  models or positive experimental    │
│                    │  test in at least one model         │
│                    ▼                                     │
│          ┌──────────────────────┐                       │
│          │      Mutagen         │                       │
│          └──────────────────────┘                       │
└─────────────────────────────────────────────────────────┘
```

Figure 3 QSAR screening scheme for mutagenicity

The suggested criterion for a positive mutagenicity prediction is that there is a positive prediction (within the AD defined in the validation) in two or more models, accepting only predictions where no significant deactivating fragments were detected. If one or more positive tests could be seen (as part of the training sets for the models) for any genotoxicity endpoint, this took precedence over model predictions.

QSAR screening algorithm for prenatal developmental toxicity
Three models predicting *in vivo* teratogenicity or fetal lethality related endpoints have been included in the screening which was performed according to the scheme illustrated in Figure 4.

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┌─────────────────────────────────────────────────────────┐
│   ┌─────────────────────────────────────────────┐       │
│   │  Assessment for developmental toxicity       │       │
│   │                                              │       │
│   │  Predictions from models for in vivo         │       │
│   │                                              │       │
│   │    •  Teratogenicity, human                  │       │
│   │    •  Drosophila melanogaster SLRL           │       │
│   │    •  Rodent dominant lethal                 │       │
│   └─────────────────────────────────────────────┘       │
│                    │                                     │
│                    │  Positive prediction or test in at  │
│                    │  least one endpoint and not         │
│                    │  predicted or tested negative for   │
│                    │  teratogenicity                     │
│                    ▼                                     │
│          ┌──────────────────────┐                       │
│          │ Developmental toxicant│                      │
│          └──────────────────────┘                       │
└─────────────────────────────────────────────────────────┘
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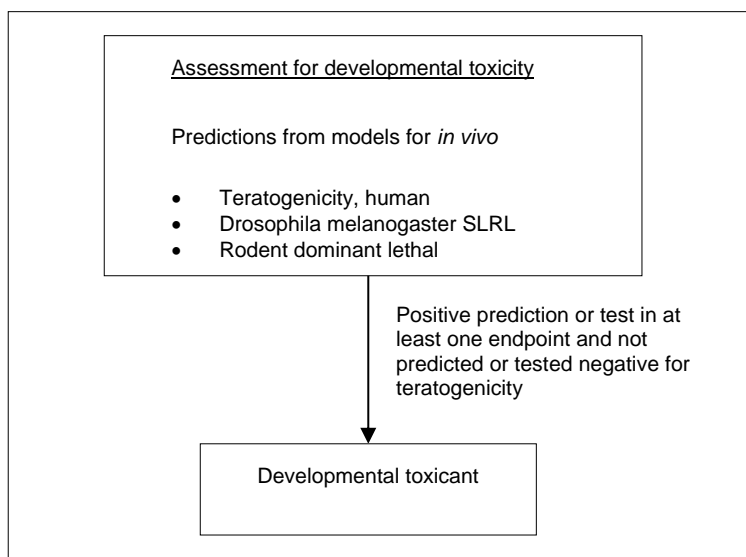
Figure 4 QSAR screening scheme for developmental toxicity.

The suggested criterion for a positive developmental toxicity prediction is a positive prediction (within the AD defined in the validation) in any of the three models and without a negative prediction in the teratogenic potential in humans model.

The QSAR models applied cover certain but far from all types of harm to the unborn child; only certain types of mechanisms causing malformation or fetal mortality are covered. Many other types of reproductive toxicity effects are known to exist – and many reproductive toxicity modes of action are currently unknown. Fertility as a reproductive toxicity endpoint was not covered.

A total of 212 REACH registered substances and 18,266 PRS not registered were flagged in this process with QSAR predictions for cancer and/or mutagenicity and/or teratogenicity and were further processed.

SPIN filter; exposure filter using SPIN information for substances not REACH registered (step 5 in figure 1)
To estimate the human exposure relevance of the identified CMR predicted substances, information from the Nordic substance register database (SPIN) was applied. The SPIN database contains non confidential information on the use of substances in products in the Nordic countries and is based on data from the product registers in Norway, Sweden, Denmark and Finland /15/.

As SPIN registrations are not required for articles, foodstuffs, cosmetics, medicinal products and substances imported/produced in quantities less than 100 kg/year per company, substances not flagged in SPIN as having human exposure relevance according to the Use Index, may still have potential human exposure relevance. Another limitation is that only chemicals used in the workplace are registered. Also, SPIN only covers use in the Nordic countries and not the whole EU. Vice versa, substances which according to SPIN have human exposure relevance may no longer be on the EU market as the information in SPIN is based on previous year's information in the national product registers. Requirements to report and update the information in the national product registers are also not identical, and information is not updated every year in all the registers.

Substances having at least one of the following SPIN parameters defined regarding use in the Nordic countries; Total Amount in tonnes, Total number of products or Range of use were submitted for further processing.

In addition, the Use Index for consumers from the Exposure toolbox of the SPIN database was required to be greater than or equal to 3, indicating a potential for direct exposure to humans. The Use Index in SPIN is an index by which exposure relevant information, of which a large proportion is confidential in the Nordic national registers, can be made publicly available in SPIN. The Use Index cannot be used to provide exact quantifications on exposure but is an indicative screening tool.

A total of 695 substances were selected.

Manual selection of cases for illustration for registered and not registered substances (step 6 in figure 1)
Within the constraints of the project it was chosen to focus on a few substance cases for illustration. The purpose was not to make comprehensive expert judgments on all the obtained QSAR prediction profiles for the 695 non-registered substances and the 212 registered substances, but to select a number of cases to illustrate how additional available information may be taken into consideration.

For the selected substances, the individual C, M and/or R predictions for key endpoints were further inspected manually to take the training set data and biophore statistical information into account. Other sources of QSAR information were also consulted, e.g. predictions from a number of Leadscope models and the PASS system for CMR properties.

Furthermore, the in-house Danish QSAR prediction database containing predictions for many endpoints covering physical-chemical properties, bioavailability, CYP P450 iso-enzyme affinity or

inhibitory effects, eco-toxicity and human health endpoints was consulted for a more holistic evaluation of the individual substances.

Finally, the eChemPortal webpage was consulted to look for possible experimental CMR related information and a quick search on Google was done to find readily available information on the occurrence and use of the substances. For the registered substances the online ECHA database with registration dossiers was furthermore consulted to see the contained experimental data /3/.

A list with 26 non-REACH-registered substances and a list with 5 REACH registered substances, both containing substances with probable significant human exposure potential and for which potential CMR properties were predicted, have been compiled. These lists illustrate how further evaluation of initially identified potential CMR substances with probable significant human exposure potential may be further evaluated for further priority setting. Such further priority setting may be used as basis for selecting substances for proposal for (harmonized) CMR-classification and / or for inclusion on the CORAP list (for targeted further confirmatory testing of CMR properties). The lists with selected illustrative cases are included in annex 5 and 6.

The resulting lists

The screening project resulted in the following lists of substances QSAR predicted to potentially have CMR properties:

1. QSAR CMR predicted PRS not registered in 2012: total list of 18,266
2. QSAR CMR predicted PRS not registered in 2012 with SPIN information on potential human exposure: total list of 695
3. QSAR CMR predicted PRS not registered in 2012, selected illustrative cases: 26
4. QSAR CMR predicted registered substances: 212
5. QSAR CMR predicted registered substances not classified for CMR (by a harmonized classification or by a self-classification of the REACH registrant), selected illustrative cases: 5

The lists are available as follows:

1. List 1_QSAR CMR predicted PRSnot registered in 2012: downloadable at www.mst.dk
2. List 2_ QSAR CMR predicted PRS not registered in 2012 with SPIN information on potential human exposure: downloadable at www.mst.dk
3. List 3_ QSAR CMR predicted PRS not registered in 2012, illustrative cases: available in Annex 5.
4. List 4_ QSAR CMR predicted registered substances: downloadable at www.mst.dk
5. List 5_ QSAR CMR predicted registered substances, illustrative cases: available in Annex 6.

It is important to note that the substances in these lists were not proven to meet the criteria for carcinogenicity, mutagenicity or reproductive toxicity. Nor has it been proven that their use constitutes a risk to consumers or workers. Further work needs to be carried out including expert assessment of all available relevant information for each substance before a conclusion on their CMR properties can be made.

These lists may be applied by the Danish EPA for prioritization purposes with the proper consultation of relevant sources to obtain possible experimental testing information on CMR properties of the substances.

Use of the lists

The results only represent positive predictions. No distinction has been made between a negative prediction for an endpoint, and an unreliable prediction (prediction outside the applicability domain of the model), which was simply discarded.

Evaluated substances which are not on the list, or substances which are on the list but without flags for either C, M or R, may have been predicted as not having this / these property(ies), or the models may not have been valid for this substance, i.e. predictions were outside the applicability domain for these models.

The lists represent QSAR-based identifications of possible CMR hazardous properties of the included substances; although SPIN information was taken into account to select chemicals with probable significant human exposure no attempt was made to evaluate the actual risk that the substances constitute in their current use in the EU.

The duty to map available information on substances lies with manufacturers / importers. The models applied in this project had according to validation results accuracies of approximately 70-85%. Substances appearing on the resulting lists may have experimental test results which are more reliable.

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Annex 1 Short introduction to QSARs

Structure-activity relationships (SARs) and quantitative structure-activity relationships (QSARs), collectively referred to as QSARs, are mathematical models that can be used to predict the physico-chemical, biological (e.g. toxicological) and environmental fate properties of molecules based on their chemical structure. "A QSAR is a mathematical model (often a statistical correlation) relating one or more quantitative parameters derived from chemical structure to a quantitative measure of a property or activity (e.g. a (eco)toxicological endpoint). QSARs are quantitative models that yield either a continuous or categorical (yes/no) result" /16/.

A QSAR model thus links information on the chemical structure of compounds with a specific property, and is subsequently used for predicting the same property for unknown compounds. Reliable predictions can be obtained for compounds that are within the domain of the developed QSAR model, i.e. for compounds that are sufficiently structurally similar to the compounds used to train the model. QSAR models are thus powerful tools for predicting chemically induced adverse effects and thus for filling data gaps.

The reliability of QSAR-predictions depends on numerous parameters relating to the mathematical methods used, the number and precision of the underlying data used for developing the model and how suitable the model is for the particular substance. In general the uncertainty of QSARs is caused predominantly by two different reasons: a) the inherent variability of the input data used to establish the model (training set); and b) the uncertainty resulting from the fact that a model can only be a partial representation of reality (in other words does not model all possible mechanisms concerning a given endpoint and does not cover all types of substances). However, as a model averages the uncertainty over all substances, it is possible for an individual model estimate to be more accurate than an individual measurement /16/.

Validation is a trial of the model performance for a set of substances independent of the training set, but within the domain of the model. The model predictions for these substances are compared with measured endpoints for the substances in order to establish the predictive performance of the model. Ideally all models should be assessed by checking how well they predict the activity of substances, which were not used to make them. This is, however, not always simple. In part valuable information may be left out by setting aside substances to be used in such an evaluation, and in part it can be extremely difficult to assess how "external" substances relate to the model's domain; that is, if they represent a random distribution within this applicability domain and thereby giving a fair picture of the predictive performance of the model.

This problem is often addressed by using one or another form of cross-validation, where a number of partial models are "externally validated" by dividing the training set into a reduced training set and a testing set. The reduced training set is used to develop a partial model, while the remaining data are used as a test set to evaluate the model predictivity. This is repeated a number of times and the results are used to calculate the predictivity measures for the models; for quantitative (continuous) models in the form of Q^2 and SDEP (standard deviation error of prediction), and for qualitative (categorical yes/no) models in the form of sensitivity (ability to correctly predict

positives), specificity (ability to correctly predict negatives) and concordance (overall accuracy, see also e.g. /16/ and /17/ for further details). In the majority of validations carried out on the models applied in this project the stable leave-many-out (LMO) cross-validation approach was used. The training set was split by random (however keeping the positive / negative balance in the subsets) into two portions of 50% of the substances, models on each of the reduced sets were made, and the one model was run to predict the training set of the other model and the other way around, repeating this 5 times. Leaving out 50% of the substances in the partial validation models is a large perturbation of the training set, which generally leads to realistic, and often pessimistic, measures of the predictivity of the model. The commercial cancer were also validated by external validation.

Concordance will vary depending on both the method used, and the endpoint in question. In general, contemporary QSAR systems can often correctly predict the activity of about 70 – 85% of the substances examined, provided that the query structures are within the domains of the models.

When applying QSAR's it is important to assure that an obtained prediction falls within the applicability domain (AD) of the models i.e., that there is sufficient similarity (in relevant descriptors) between the query substance and substances in the training set of the model. There is no single and absolute applicability domain for a given model /16/. Generally, the broader the applicability domain is defined the lower predictivity can be expected. The applicability domain should be clearly defined and the validation results should correspond to this defined domain, which is again used when the model is applied for predictions.

QSARs tools are used more and more by authorities e.g. in the US and the EU, as well as by industry, to assess physico-chemical, (eco-)toxicological, ADME, MoA, and environmental fate properties of substances.

In the new EU chemicals legislation, REACH, all other options, including use of (Q)SARs, should be considered before performing (or requiring) vertebrate testing /1/. Annex XI of REACH contains the following wording regarding (Q)SARs:

Results obtained from valid qualitative or quantitative structure-activity relationship models ((Q)SARs) may indicate the presence or absence of a certain dangerous property. Results of (Q)SARs may be used instead of testing when the following conditions are met:

Results are derived from a (Q)SAR model whose scientific validity has been established,

The substance falls within the applicability domain of the (Q)SAR model,

Results are adequate for the purpose of classification and labelling and/or risk assessment, and,

Adequate and reliable documentation of the applied method is provided.

Annex 2 Structure set preparation for QSAR screening

It is a prerequisite for using QSAR software to generate predictions that *structural information* in the format of e.g. SMILES or sdf is available. As structural information was not required as part of the pre-registration and as no formal structure set on the PRS from ECHA exists, other sources for the structural information were searched.

The Computational Toxicology Group within the Joint Research Centre (JRC) has generated structure information for PRS by using the ACDLabs Name-To-Structure software and validated them with a random sample /9/. In total 80,413 structures were generated, including both discrete organics, inorganics etc.

The SDF records of the original Daginnus set were converted into an OASIS Database Manager database using OASIS Database Manager 1.7.3 and removing the small number of invalid records and duplicates found in the input files; a total of 80,394 substances were imported.

The QSAR software applied in the screening can handle organic substances with an unambiguous structure, i.e. so-called discrete organics (no mixtures/UVCB's, organometallics, inorganics), which are:

- Containing at least two carbon atoms
- Containing only H, Li, B, C, N, O, F, Na, Mg, Si, P, S, Cl, K, Ca, Br, or I
- In reality not mixtures with two or more "big components" when hydrolysed

Therefore, subsequent processing included hydrolysis simulation, exclusion of inorganic substances as well as substances with only one carbon atom, mixtures, generic structures, structures containing heavy and other inacceptable atoms or inappropriate ions, and structures with valency errors:

Database Manager hydrolysis simulator generates, among others, the following flags and parameters:
MOL._WEIGHT: if MOL._WEIGHT is not defined, the structure may be incorrect or too complex.
Calc_Trace_Wrong_SMILES: The SMILES has minor problems
Calc_Trace_Too_Long: The SMILES is too long

Combinations of these:
No Calc_Trace warnings and MOL._WEIGHT is defined: The structure is OK for Database Manager
No Calc_Trace warnings and MOL._WEIGHT is not defined: The structure contains a generic atom ('R') or cannot be interpreted by Database Manager

Calculation SMILES was generated stripping off ions for substances where this was found appropriate, in order to facilitate the QSAR prediction of these substances:

Calculation SMILES generation assigns the following mutually exclusive flags:
Calc_OK_Discrete - calculation SMILES generated OK and the structure is discrete
Calc_OK_Mixture - calculation SMILES generated OK, the structure can be a mixture
none - the calculation SMILES is not ok

In order to exclude low quality structures and/or ones containing generic atoms, structures flagged with 'SMILES too long' and structures flagged with 'Calc_Trace warning' AND 'MOL._WEIGHT not defined' were removed. These filterings resulted in 72,308 structures.

Finally, structures that were ok for the substance database platform but not accepted by the QSAR prediction software (MultiCASE) were removed.

The total number of substances suitable for prediction was 70,983.

Annex 3 MultiCASE

MultiCASE is a commercial artificial intelligence software system. In the creation of a model, the program analyses a training set of substances with known activity and starts by dividing each substance into fragments containing 2–10 interconnected atoms (non-hydrogen atoms). These fragments are labelled with the experimental value of the parent substance. MultiCASE determines the distribution of all fragments among the substances in the training set. The distribution of the fragments is assumed to be binomial. If a fragment is over-represented (p>95%) in the group of active or inactive substances the fragment is assumed to be relevant for the modelled activity. If the fragment is not significantly overrepresented in active or inactive substances it will not be considered important. A fragment with a statistical correlation to active or inactive substances is called a biophore or a biophobe, respectively. When all fragments have been examined for their importance to activity, a hierarchical selection takes place, starting with the biophore with the most statistically significant result. Substances containing this substructure are set aside and the next biophore is found in the same manner. This is repeated until either the entire training set is used or there are no more statistically significant fragments. The whole procedure is then performed for biophobes in an identical way. Each group of substances containing a biophore or biophobe is then analysed to find modulators that either enhance or decrease the probability of the fragment being a biophore/biophobe. The modulators can be structural fragments or chemical properties (e.g. activating fragments, deactivating fragments, log K_{ow}, molecular orbital energies, volume / surface descriptors) /18/.

When a model is developed and MultiCASE predicts the activity for a substance, the program first looks for biophores contained in the substance. If it identifies a biophore it then looks for modulators to calculate the activity of the substance. When MultiCASE predicts the activity of substances it produces a system of output values, which can be further processed to define unambiguous positive, negative and equivocal outcomes. Positive means that the model predicts that if this substance would be tested, e.g. in the Ames test, the results would likely be positive for Ames mutations, i.e. the substance would be an Ames mutagen. The opposite goes for the negative predictions. Equivocal means that a definite call could not be made, as it can also happen in experimental tests. An equivocal was not seen as reliable and was not used in the cross-validation.

The applicability domains for MultiCASE models as defined by the US Food and Drug Administration (FDA) /14/ and implemented in the MultiCASE software were used in this project. This means that no warnings in the predictions were accepted, except warning for one unknown fragment in substances where a significant biophore had been detected. A compound was only predicted as positive/active if it contained at least one statistically significant biophore. Only positive predictions where no significant deactivating fragments were detected were accepted.

The data was entered into MultiCASE using SMILES notations (without stereochemical information).

Annex 4 Details on individual models

Reverse mutation test, Ames *in vitro* (internal id: AGA)
- Endpoint: The bacterial reverse mutation test detects point mutations, which involve substitution, addition or deletion of one or a few DNA base pairs, as described in the OECD test guideline 471.
- Source: Developed by the DTU Food QSAR group in the MultiCASE software based on data from Kazius et al. /19/
- Technical data: The training set consists of 4102 substances, 2299 positive and 1803 negative. Cross-validation by 5 times twofold 50% gave: sensitivity 84%, specificity 83% and concordance 84%.

Chromosome aberration in CHO (Chinese Hamster Ovary) *in vitro* (internal id: A61)
- Endpoint: The chromosome aberration tests identifies agents that cause structural chromosome aberrations in cultured mammalian cells, as described in the OECD test guideline 473.
- Source: Commercial MultiCASE model A61 /20/
- Technical data: The training set consists of 233 substances, 95 positive, 4 marginal and 134 negative. DTU cross-validation by 5 times twofold 50% gave: sensitivity 32%, specificity 91% and concordance 70%.

Chromosome aberration in CHL (Chinese Hamster Lung) *in vitro* (internal id: AN6)
- Endpoint: The chromosome aberration tests identifies agents that cause structural chromosome aberrations in cultured mammalian cells, as described in the OECD test guideline 473. Chromosome damage is expressed as breakage of single or both chromatids, sometimes followed by reunion between chromatids or of both chromatids at an identical site.
- Source: Developed by the DTU Food QSAR group in the MultiCASE software, and based on data from Sofuni /21, 22/ and described in /23/.
- Technical data: The training set consists of 600 substances, 294 positive and 306 negative. Cross-validation by 5 times twofold 50% gave: sensitivity 58%, specificity 87% and concordance 74%.

Mouse lymphoma *in vitro;* Mammalian Cell Gene Mutation Test measured by TK (internal id: AN1)
- Endpoint: The mammalian cell gene mutation test in mouse lymphoma cells detects mutations affecting the heterozygous thymidine kinase (TK) locus, as described in the OECD. It identifies substances acting as clastogens (delete, add, or rearrange chromosome sections) as well as point mutagens.
- Source: Developed by the DTU Food QSAR group in the MultiCASE software, and based on data from /24/.
- Technical data: The training set consists of 555 substances, 282 positive, 22 marginal and 251 negative. Cross-validation by 5 times twofold 50% gave: sensitivity 69%, specificity 86% and concordance 79%.

Rodent dominant lethal *in vivo* (internal id: AN4)
- Endpoint: The rodent dominant lethal test identifies major genetic damage, mainly the induction of structural and numerical chromosomal anomalies, as described in the OECD test guideline 478. Early embryonic deaths is the most significant index of dominant lethality and as such used as endpoint
- Source: Developed by the DTU Food QSAR group in the MultiCASE software, and based on data from /25/.

- Technical data: The training set consists of 191 substances, 78 positive and 113 negative. Cross-validation by 5 times twofold 50% gave: sensitivity 41%, specificity 95% and concordance 76%.

Sex-Linked Recessive Lethal test in Drosophila melanogaster *in vivo* (internal id: AN5)
- Endpoint: The Drosophila melanogaster SLRL (Sex-Linked Recessive Lethal) test detects the occurrence of mutations, point mutations and small deletions, in the germ line of the insect, as described in the OECD test guideline 477.
- Source: Developed by the DTU Food QSAR group in the MultiCASE software, and based on data from /26/.
- Technical data: The training set consists of 377 substances, 190 positive and 187 negative. Cross-validation by 5 times twofold 50% gave: sensitivity 74%, specificity 88% and concordance 82%.

SCE Mouse *in vivo* (internal id: ANB)
- Endpoint: The Mouse SCE (Sister Chromatid Exchange) assay detects interchange of DNA between two sister chromatids of a duplicating chromosome.
- Source: Developed by the DTU Food QSAR group in the MultiCASE software, and based on data from /27/.
- Technical data: The training set consists of 265 substances, 103 positive and 162 negative. Cross-validation by 5 times twofold 50% gave: sensitivity 71%, specificity 87% and concordance 86%.

Mouse mammalian bone marrow erythrocyte micronucleus *in vivo* (internal id: ANC)
- Endpoint: The Mouse micronucleus assay detects micronuclei produced by damage to the chromosomes or the mitotic apparatus in red blood cells, as described in the OECD test guideline 474.
- Source: Developed by the DTU Food QSAR group in the MultiCASE software, and based on data from /28-31/.
- Technical data: The training set consists of 358 substances, 168 positive and 190 negative. Cross-validation by 5 times twofold 50% gave: sensitivity 30%, specificity 85% and concordance 66%.

Comet assay *in vivo* (internal id: ANE)
- Endpoint: The Comet assay detects DNA strand break and can be applied to virtually any organ of interest.
- Source: Developed by the DTU Food QSAR group in the MultiCASE software, and based on data from /32/ plus a number of physiological substances theoretically assumed not to have the effect (such as various amino acids, sugar molecules, fatty acids etc.), which were included to get a better distribution between positives and negatives in the training set for the model.
- Technical data: The training set consists of 286 substances, 136 positive and 150 negative. Cross-validation by 5 times twofold 50% gave: sensitivity 63%, specificity 93% and concordance 84%.

FDA cancer models *in vivo*
- Endpoint: The cancer test development of tumours, as described in the OECD test guideline 451. In the experimental test, the test substance is administered by an appropriate route to the animals for a major portion of their lifespan. The highest dose level should elicit signs of toxicity, without substantially altering the normal lifespan due to effects other than tumours.
- Source: Commercial MultiCASE open models AF1-AF4 and models including proprietary data AG1-AG4, and based on data from the NTP (US National Toxicology Program) rodent carcinogenicity database, the Lois Gold Carcinogen Potency Database, FDA/CDER (US Food and Drug Administration / Center for Drug Evaluation and Research) archives, and the scientific literature. The proprietary (confidential) data constitute around ten percent of the training sets in AG1-AG4. The open models are solely based on the non-proprietary data part /33/.
- Technical data:
- AG1: Male Rat incl. proprietary data: The training set consists of 1381 substances. Cross-validation cannot be performed as part of the training set is hidden for the user. (The open part of the model, corresponding to the model AF1 was cross-validated: The training set consists of 1277 substances, 590 positive, 92 marginal and 595 negative. DTU cross-

validation by 5 times twofold 50% gave: sensitivity 44%, specificity 77% and concordance 69%.)

- AG2: Female Rat incl. proprietary data: The training set consists of 1376 substances. Cross-validation cannot be performed as part of the training set is hidden for the user. (The open part of the model, corresponding to the model AF2 was cross-validated: The training set consists of 1274 substances, 546 positive, 64 marginal and 664 negative. DTU cross-validation by 5 times twofold 50% gave: sensitivity 42%, specificity 83% and concordance 74%.)
- AG3: Male Mouse incl. proprietary data: The training set consists of 1252 substances. Cross-validation cannot be performed as part of the training set is hidden for the user. (The open part of the model, corresponding to the model AF3 was cross-validated: The training set consists of 1157 substances, 495 positive, 67 marginal and 595 inactive. DTU cross-validation by 5 times twofold 50% gave: sensitivity 47%, specificity 83% and concordance 76%.)
- AG4: Female Mouse incl. proprietary data: The training set consists of 1263 substances. Cross-validation cannot be performed as part of the training set is hidden for the user. (The open part of the model, corresponding to the model AF4 was cross-validated: The training set consists of 1169 substances, 526 positive, 58 marginal and 585 inactive. DTU cross-validation by 5 times twofold 50% gave: sensitivity 42%, specificity 82% and concordance 74%.)
- The total suite of models were validated by external validation with 100 substances which gave overall; sensitivity 59%, specificity 98% and concordance 75%
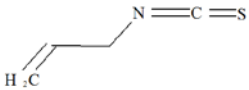
RCA call

- For a substance to be positive according to the RCA methodology there have to be two or more positive carcinogenicity predictions within AG1-4, where only predictions for substances without significant deactivating fragments are accepted /14/.

Teratogenicity FDA TERIS (internal id: A49)

- Endpoint: Teratogenic potential in humans
- Source: Commercial MultiCASE model A49 based on data from the US Teratogen Information System (TERIS) and a database based on the US Food and Drug Administration guidelines/34/.
- Technical data: The training set consists of 323 substances, 130 positives and 193 negatives. DTU cross-validation by 5 times twofold 50% gave: sensitivity 50%, specificity 91% and concordance 79%.

Annex 5 QSAR CMR predicted PRS not registered in 2012, illustrative cases
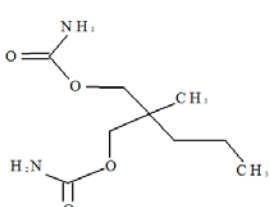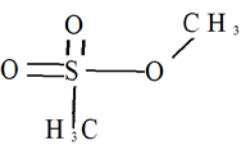
Identity	QSAR CMR[1] – Danish QSAR Database	QSAR predictions – other models[2]	Use[3], exposure & C&L	eChemPortal[4]
1-Propene, 3-isothiocyanato- (allyl isothiocyanate) CAS RN 57-06-7 N=C=S structure	CMR Based on positive predictions in models for Ames (part of the training set), CA CHO (part of training set), Drosophila m. SLRL (part of training set), and FDA RCA cancer call based on male rat (part of training set) and female rat (part of training set).	Cancer Benigni-Bossa carcinogenicity (TOXTREE, v.1.0.0) = positive (good reliability) Mutagenicity CAESAR mutagenicity model (v.2.1.12) = positive (good reliability) T.E.S.T mutagenicity consensus model = positive Benigni-Bossa mutagenicity (TOXTREE, v.1.0.0) = positive (good reliability) Reproductive toxicity T.E.S.T developmental toxicity consensus model = positive	Use: Naturally in seeds from mustard and produced synthetically. Used e.g. as pesticide, as a flavouring agent in foods (on EU flavouring substances list), as a fumigant, in ointments and mustard plasters and as a military poison gas. SPIN: Narrow range of applications in DK (4-10) (last record from 2010). One or several uses indicate a potential for direct exposure to humans (consumer and occupational). Total tonnage in DK: 0.2 Tonnes (last record from 2003). ECHA C&L Inventory: Total number of notifiers: >500. No self-classifications for CMR.	NTP: Carcinogenic in male rats and equivocal in female rats. Not carcinogenic in mice of either sex (MTD probably not reached). IARC: not classifiable as to its carcinogenicity to humans (Group 3), limited evidence for the carcinogenicity to experimental animals. Not teratogenic to mice, rats, hamsters or rabbits, but resorptions were seen in mice and rats. US NJ RTK Hazardous Substance Fact Sheet; may damage the developing foetus. NTP: In vivo; positive SCE, negative Drosophila SLRL and negative/equivocal CA; in vitro; positive CA CHO, ML and SCE, and weakly positive/negative Ames.

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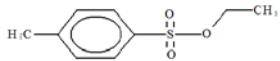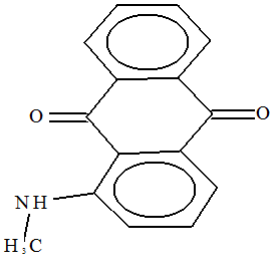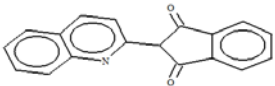
[1] The underlying QSAR models for the resulting positive C, M or R call are listed. QSAR models in the CMR algorithms which are not mentioned may either have given negative predictions or the chemical may have been out of the applicability domain. No information is included about the manual evaluations of the predictions or information from other CMR relevant models and systems applied.
[2] Predictions generated in CMR models in CAESAR, TOXTREE, SarPy and T.E.S.T. Obtained predictions with reported low reliability or outside applicability domain are not reported.
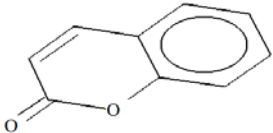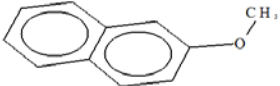[3] Quick Google searches and the US EPA ACTOR site primarily used to find information.
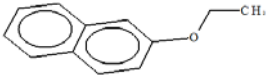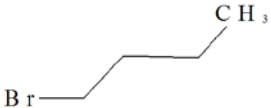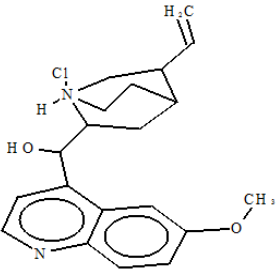[4] The experimental results listed in this column are the non-comprehensive results of a quick look-up.

Identity	QSAR CMR[1] – Danish QSAR Database	QSAR predictions – other models[2]	Use[3], exposure & C&L	eChemPortal[4]
1,3-Propanediol, 2-methyl-2-propyl-, dicarbamate (Meprobamate) CAS RN 57-53-4	R Based on positive prediction/test in model for human teratogenicity (part of training set).	CAESAR developmental toxicity model (v.2.1.6) = positive (good reliability) T.E.S.T developmental toxicity consensus model = Positive (part of training set)	Use: Anxiolytic drug launched in 1955. EMA has recommended suspension in 2012 due to serious side effects (confusion, loss of consciousness). SPIN: Not recorded in DK. Very narrow range of applications in SE (1-3) (last record from 2010). One or several uses indicate a potential for direct exposure to humans (consumer and occupational). Total tonnage in SE: not recorded. ECHA C&L Inventory: Total number of notifiers: 26. No self-classifications for CMR.	UMD list; recognized developmental toxicant.
Methanesulfonic acid, methyl ester (Methyl methanesulfonate) CAS RN 66-27-3	CMR Based on positive predictions in models for Ames (part of training set), CA CHL (part of training set), mouse lymphoma (part of training set), Drosophila m. SLRL (part of training set), mouse micronucleus (part of training set), Rodent dominant lethal (part of training set), mouse SCE (part of training set), mouse Comet (part of training set), and FDA cancer	Cancer No predictions within AD obtained Mutagenicity CAESAR mutagenicity model (v.2.1.12) = positive (good reliability) T.E.S.T mutagenicity consensus model = positive (part of training set) Reproductive toxicity T.E.S.T developmental toxicity consensus model = positive	Use: Research chemical SPIN: Intermediate range of applications in DK (11-32) (last record from 2010). One or several uses indicate a very probable direct exposure to humans (consumer and occupational). Total tonnage in DK: not recorded. ECHA C&L Inventory: Total number of notifiers: 29. 24 have self -classified as carc. 1 B 1has self-classified as	US EPA ACTOR: An alkylating agent in cancer therapy that may also act as a mutagen by interfering with and causing damage to DNA. CPDB Mouse TD50: 31.8 mg/kg. IARC; Increased frequency of resorptions and congenital malformations. Induced mouse germ cell mutations and chromosomal aberrations, and DNA damage, micronuclei, sister chromatid exchanges and chromosomal aberrations in somatic cells of rodents *in vivo.* Increased the frequency of DNA damage, gene

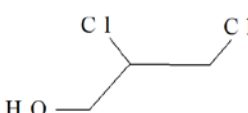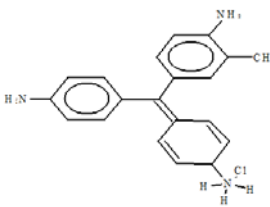| Identity	QSAR CMR[1] – Danish QSAR Database	QSAR predictions – other models[2]	Use[3], exposure & C&L	eChemPortal[4]
	male mouse (part of training set).		Mut 1B, Carc 1b & Rep 2 • 1 has self-classified as Mut 2 & Carc 1 A • 3 have not self-classified for CMR	mutation, sister chromatid exchanges and micronuclei in human and rodent cell cultures, as well as chromosomal aberrations in rodent cells *in vitro*. Induced somatic and sex-linked mutations in *Drosophila*. Was mutagenic in bacteria. Is *probably carcinogenic to humans (Group 2A)*
Benzenesulfonic acid, 4-methyl-, ethyl ester (ethyl toluene-4-sulphonate) CAS RN 80-40-0	MR Based on positive predictions in models for Drosophila m. SLRL, mouse micronucleus, mouse SCE, and mouse Comet.	Mutagenicity CAESAR mutagenicity model (v.2.1.12) = positive (good reliability) SarPy model (v.1.0.6) = positive (good reliability) T.E.S.T mutagenicity consensus model = positive (part of training set) Benigni-Bossa mutagenicity (TOXTREE, v.1.0.0) = negative (good reliability) Reproductive toxicity T.E.S.T developmental toxicity consensus model = positive	Use: No information found SPIN: Not recorded in DK. Very narrow range of applications in SE (1-3) (last record from 2010). No indication of exposure to humans. Total tonnage in SE: not recorded. ECHA C&L Inventory: Total number of notifiers: 38. No self-classifications for CMR.	Gene-tox: Positive Ames and other in vitro tests (SHE cell transformation, DNA repair, Human UDS), negative in vitro forward and reverse gene mutation. CCRIS; negative / weakly positive Ames, and positive ML and MN in vitro.
9,10-Anthracenedione, 1-(methylamino)-(1-(methylamino)anthraquinone) CAS RN 82-38-2	CM Based on positive predictions in models for Ames, mouse lymphoma, Mouse SCE, mouse Comet, and FDA RCA cancer call based on male rat	Cancer CAESAR carcinogenicity model (v.2.1.8) = positive (good reliability) Benigni-Bossa carcinogenicity (TOXTREE, v.1.0.0) = positive (good	Use: Dye known as disperse red 9. Used in coloured smoke and in manufacture of rubber and plasstic products. SPIN: Very narrow range of applications in DK (1-3) (last	No CMR relevant information has been identified.

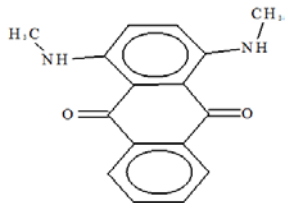| Identity	QSAR CMR[1] – Danish QSAR Database	QSAR predictions – other models[2]	Use[3], exposure & C&L	eChemPortal[4]
	and female rat.	reliability) Mutagenicity CAESAR mutagenicity model (v.2.1.12) = positive (good reliability) SarPy model (v.1.0.6) = positive (good prediction) T.E.S.T mutagenicity consensus model = positive Benigni-Bossa mutagenicity (TOXTREE, v.1.0.0) = positive (good reliability)	record from 2010). One or several uses indicate a potential for direct exposure to consumers and a very probable potential for occupational exposure. Total tonnage in DK: 1 Tonne (last record from 2007). ECHA C&L Inventory: Total number of notifiers: >1000. No self-classifications for CMR.	
1H-Indene-1,3(2H)-dione, 2-(2-quinolinyl)- CAS RN 83-08-9 	CR Based on positive predictions in models for Ames (part of training set), CA CHL, mouse lymphoma (part of training set), FDA RCA cancer call based on male rat (part of training set) and female rat (part of training set), and human teratogenicity.	Cancer Benigni-Bossa carcinogenicity (TOXTREE, v.1.0.0) = negative (low reliability) – however, part of training set (dataset ID: 681), experimental value = positive Reproductive toxicity CAESAR developmental toxicity model (v.2.1.6) = positive (good reliability) T.E.S.T developmental toxicity consensus model = positive	Use: Pigment. Used in industrial coatings, plastics, decorative paints. SPIN: Not recorded in DK. Very narrow range of applications in NO (1-3) (last record from 2010). One or several uses indicate a probable exposure to humans. Total tonnage in NO: not recorded. ECHA C&L Inventory: No records	No CMR relevant information has been identified (except as mentioned in previous columns the experimental data in the model training sets).

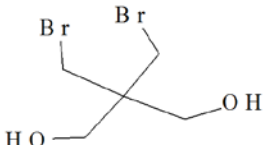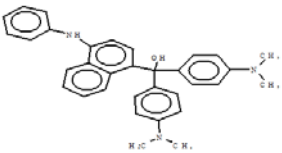| Identity	QSAR CMR[1] – Danish QSAR Database	QSAR predictions – other models[2]	Use[3], exposure & C&L	eChemPortal[4]
2H-1-Benzopyran-2-one (coumarin) CAS RN 91-64-5 	CMR Based on positive predictions in models for Ames (part of training set), CA CHO (part of training set), mouse lymphoma, mouse micronucleus, mouse SCE, FDA RCA cancer call based on male rat (part of training set), female rat (part of training set), male mouse (part of training set) and female mouse (part of training set), and human teratogenicity (part of training set).	Cancer CAESAR carcinogenicity model (v.2.1.8) = positive (domain warning) Benigni-Bossa carcinogenicity (TOXTREE, v.1.0.0) = positive (good reliability) (part of training set) Mutagenicity CAESAR mutagenicity model (v.2.1.12) = positive (good reliability) (part of training set) SarPy model (v.1.0.6) = negative (low reliability) (part of training set. Experimental value = positive) T.E.S.T mutagenicity consensus model = negative (part of training set. experimental value = positive) Benigni-Bossa mutagenicity (TOXTREE, v.1.0.0) = positive (good reliability) Reproductive toxicity CAESAR developmental toxicity model (v.2.1.6) = positive (good reliability (part of training set) T.E.S.T developmental toxicity consensus model = negative (part of training set. experimental value = positive)	Use: Fragrant found in many plants. Used as flavouring agent in food, cosmetics and tobacco, anticoagulant precursor. SPIN: Very wide range of applications in DK (>100) (last record from 2010). One or several uses indicate a very probable exposure to humans (consumer and occupational). Total tonnage in DK: 0.1 Tonnes (last record 2010). However, 38 Tonnes are reported from SE (2009). ECHA C&L Inventory: Total number of notifiers: >1000. • 86 notifiers have self-classified as Carc 2 • The remaining notifiers have not self-classified for CMR	CPDB Rat TD50 39.2 mg/kg/d, Mouse TD50 103 mg/kg/d. IARC; limited evidence in experimental animals for carcinogenicity; not classifiable as to its carcinogenicity to humans (Group 3), and no signs of teratogenicity in mice, rats, rabbits or miniature pigs. UMD; listed as a teratogen and a mutagen in the "Dangerous Properties of Industrial Materials", 7th Ed., by N. Irving Sax and Richard J. Lewis. NTP; negative in Drosophila SLRL and MN in vivo (peripheral blood), positive in Ames and positive in vitro in CHO SCE, and weakly positive in vitro in CHO CA.

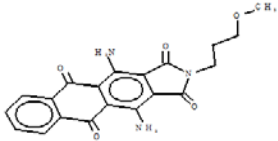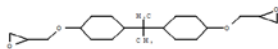| Identity	QSAR CMR[1] – Danish QSAR Database	QSAR predictions – other models[2]	Use[3], exposure & C&L	eChemPortal[4]
Naphthalene, 2-methoxy- CAS RN 93-04-9 	M Based on positive predictions in models for mouse micronucleus, mouse SCE, and mouse Comet.	CAESAR mutagenicity model (v.2.1.12) = positive (domain warning) T.E.S.T mutagenicity consensus model = positive	Use: Impurity of Naproxen SPIN: Very wide range of applications in DK (>100) (last record from 2010). One or several uses indicate a very probable exposure to humans (consumer and occupational). Total tonnage in DK: < 0.1 Tonnes (last record 2010). ECHA C&L Inventory: Total number of notifiers >1000. No self-classifications for CMR.	No CMR relevant information has been identified.
Naphthalene, 2-ethoxy- CAS RN 93-18-5 	M Based on positive predictions in models for mouse micronucleus, mouse SCE, and mouse Comet.	CAESAR mutagenicity model (v.2.1.12) = positive (domain warning) SarPy model (v.1.0.6) = negative (domain warning) T.E.S.T mutagenicity consensus model = positive	Use: No information found SPIN: Very wide range of applications in DK (>100) (last record from 2010). One or several uses indicate a very probable exposure to humans (consumer and occupational). Total tonnage in DK: 0.1 Tonnes (last record 2010). ECHA C&L Inventory: Total number of notifiers >1000. No self-classifications for CMR.	CCRIS: Negative Ames.
Butane, 1-bromo- CAS RN 109-65-9	C Based on positive predictions in models for Ames, mouse	CAESAR carcinogenicity model (v.2.1.8) = negative (domain warning)	Use: No information found SPIN: Not recorded in DK. Very narrow range of applications in SE	CCRIS: Positive Ames, negative MN in vitro.

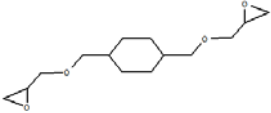| Identity	QSAR CMR[1] – Danish QSAR Database	QSAR predictions – other models[2]	Use[3], exposure & C&L	eChemPortal[4]
	lymphoma, and FDA RCA cancer call based on male rat, female rat, male mouse and female mouse.		(1-3) (last record from 2010). One or several uses indicate a potential exposure to consumers and a probable exposure to workers. Total tonnage in SE: confidential (an old record exist with 10 tonnes in SE in 1999). ECHA C&L Inventory: Total number of notifiers: 129. No self-classifications for CMR.	
Cinchonan-9-ol, 6'-methoxy-, monohydrochloride, (8alpha,9R)- (Quinine hydrochloride) CAS RN 130-89-2 	MR Based on positive predictions in models for Drosophila m. SLRL, mouse SCE (part of training set), mouse Comet, and human teratogenicity (part of training set).	Mutagenicity No predictions within AD obtained Reproductive toxicity No predictions within AD obtained	Use: Naturally in cinchona tree bark and produced synthetically. Used as antimalarial,analgesic,anti-inflammatory; flavouring agent in beverages. SPIN: Not recorded in DK. Very narrow range of applications in SE (1-3) (last record from 2010). One or several uses indicate a potential exposure to consumers and workers. Total tonnage in SE: confidential. ECHA C&L Inventory: Total number of notifiers: 798. No self-classifications for CMR.	GENE-TOX: MN (Mammalian polychromatic erythrocytes) in vitro positive, SCE in vivo positive. CCRIS: Positive Ames (E.Coli). EDSPDB Reproductive Toxicity Ranking; limited study showed teratogenic effects in rats.

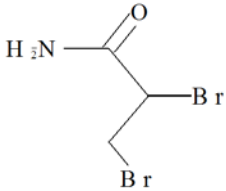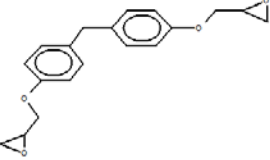| Identity	QSAR CMR[1] – Danish QSAR Database	QSAR predictions – other models[2]	Use[3], exposure & C&L	eChemPortal[4]
Benz[b]indeno[1,2-d]pyran-3,4,6a,9,10(6H)-pentol, 7,11b-dihydro-, cis-(+)- (Haematoxylin) CAS RN 517-28-2	CR Based on positive predictions in models for CA CHL, FDA RCA cancer call based on male rat (part of training set), female rat (part of training set), and human teratogenicity.	<u>Cancer</u> CAESAR carcinogenicity model (v.2.1.8) = positive (good reliability) (part of training set, experimental value = positive) Benigni-Bossa carcinogenicity (TOXTREE, v.1.0.0) = negative (low reliability), however, part of training set, experimental value = positive) <u>Reproductive toxicity</u> CAESAR developmental toxicity model (v.2.1.6) = positive (domain warning) T.E.S.T developmental toxicity consensus model = positive	Use: Extracted from logwood tree. Used as histology stain. SPIN: Very narrow range of applications in DK (1-3) (last record from 2010). One or several uses indicate a potential for direct exposure to consumers and a very probable potential for occupational exposure. Total tonnage in DK: confidential (last record from 2010). ECHA C&L Inventory: Total number of notifiers: 33. No self-classifications for CMR.	CPDB: Rat TD50 1000 mg/kg/d (hmo(B)), no test in mouse. CCRIS: Negative Ames tests.
Benzene, 1-bromo-4-(bromomethyl)- (alpha,p-Dibromotoluene) CAS RN 589-15-1	C Based on positive predictions in models for mouse lymphoma, FDA RCA cancer call based on male rat , female rat, male mouse, and female mouse.	No predictions within AD obtained	Use: No information found SPIN: Not recorded in DK. Very narrow range of applications in NO (1-3) (last record from 2010). One or several uses indicate a potential exposure to consumers. Total tonnage in NO: confidential. ECHA C&L Inventory: Total number of notifiers: 38. No self-classifications for CMR.	CCRIS: Negative Ames tests.
1-Propanol, 2,3-dichloro-	C Based on positive predictions	No predictions within AD obtained	Use: Production of epichlorohydrin	No CMR relevant information has been identified.

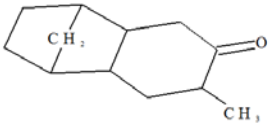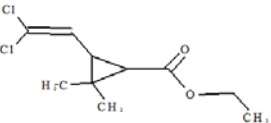| Identity	QSAR CMR[1] – Danish QSAR Database	QSAR predictions – other models[2]	Use[3], exposure & C&L	eChemPortal[4]
CAS RN 616-23-9 C l C l H O	in models for Ames (part of training set), CA CHO, CA CHL (part of training set), mouse lymphoma, and FDA RCA cancer call based on female rat, male mouse and female mouse.		SPIN: Very narrow range of applications in DK (1-3) (last record from 2010). One or several uses indicate a potential exposure to workers. Total tonnage in DK: confidential. ECHA C&L Inventory: Total number of notifiers: 53. • 1 self-classification for Mut 2 • 52 notifiers without self-classifications for CMR	
Benzenamine, 4-[(4-aminophenyl)(4-imino-2,5-cyclohexadien-1-ylidene)methyl]-2-methyl-, monohydrochloride (Magenta I) CAS RN 632-99-5	C Based on positive predictions in models for Ames (part of training set), mouse lymphoma, and FDA RCA cancer call based on female rat, male mouse and female mouse.	No predictions within AD obtained	Use: Magenta dye. Textile dye; bacteria stain. SPIN: Very narrow range of applications in DK (1-3) (last record from 2010). One or several uses indicate a potential exposure to consumers and workers. Total tonnage in DK: <0.1 Tonnes (last record from 2010). ECHA C&L Inventory: Total number of notifiers: 103. • 3 have self-classified as Carc. 1 A • 1 has self-classified as Carc. 1 B	IARC: Magenta containing CI Basic Red 9 is *possibly carcinogenic to humans (Group 2B)*.

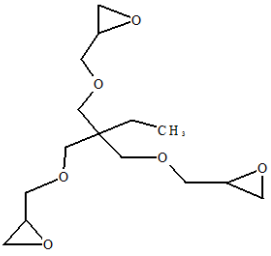| Identity	QSAR CMR[1] – Danish QSAR Database	QSAR predictions – other models[2]	Use[3], exposure & C&L	eChemPortal[4]
			• 61 have self-classified as Carc. 2 • 38 have not self-classified for CMR	
9,10-Anthracenedione, 1,4-bis(methylamino)- CAS RN 2475-44-7 	CM Based on positive predictions in models for Ames, mouse lymphoma, mouse SCE, mouse Comet, and FDA RCA cancer call based on male rat and female rat.	Cancer CAESAR carcinogenicity model (v.2.1.8) = positive (good prediction) Benigni-Bossa carcinogenicity (TOXTREE, v.1.0.0) = positive (good prediction) Mutagenicity CAESAR mutagenicity model (v.2.1.12) = positive (good reliability) SarPy model (v.1.0.6) = positive (good reliability) T.E.S.T mutagenicity consensus model = positive Benigni-Bossa mutagenicity (TOXTREE, v.1.0.0) = positive (good prediction)	Use: No information found SPIN: Very narrow range of applications in DK (1-3). Narrow range of applications in SE (4-10) (last record from 2010). One or several uses indicate a probable exposure to consumers and workers. Total tonnage in DK: <0.1 Tonnes (last record from 2010). ECHA C&L Inventory: Total number of notifiers: 47. No self-classifications for CMR.	No CMR relevant information has been identified.
1,3-Propanediol, 2,2-bis(bromomethyl)- CAS RN 3296-90-0	C Based on positive predictions in models for Ames (part of training set), mouse lymphoma, and FDA RCA	CAESAR carcinogenicity model (v.2.1.8) = positive (good prediction) (part of training set, experimental value = positive) Benigni-Bossa carcinogenicity	Google: Flame retardant SPIN: Very narrow range of applications in DK (1-3). Narrow range of applications in SE (4-10) (last record from 2010). One or	HPVIS: Positive Ames, CA in vitro and MN in vivo; negative SCE in mammalian cells in vitro. Increased tumor incidence at multiple sites in rats and mice. Female-specific decrease in reproductive capacity in mice. IARC: Possibly carcinogenic to humans

Identity	QSAR CMR[1] – Danish QSAR Database	QSAR predictions – other models[2]	Use[3], exposure & C&L	eChemPortal[4]
	cancer call based on male rat (part of training set), female rat (part of training set), male mouse (part of training set), and female mouse (part of training set).	(TOXTREE, v.1.0.0) = positive (good prediction) (part of training set, experimental value = positive)	several uses indicate a potential exposure to consumers (DK) and a very probable exposure to workers (SE). Total tonnage in DK: confidential. ECHA C&L Inventory: Total number of notifiers: 116. • 85 have self-classified as Carc 1B and Mut 1B • 30 have self-classified as Carc 2 • 1 has not classified for CMR	(Group 2B)
1-Naphthalenemethanol, alpha,alpha-bis[4-(dimethylamino)phenyl]-4-(phenylamino)- (Solvent Blue 4) CAS RN 6786-83-0 	MR Based on positive predictions in models for rodent dominant lethal mouse SCE and mouse Comet.	Mutagenicity T.E.S.T mutagenicity consensus model = positive Reproductive toxicity T.E.S.T developmental toxicity consensus model = positive	Use: Used in inks and dyes SPIN: Very narrow range of applications in DK & SE (1-3) (last record from 2010). One or several uses indicate a probable exposure to workers (DK) and a very probable exposure to consumers and workers (SE). Total tonnage in DK: confidential. Total tonnage in SE: 1 Ton (last record from 2010). ECHA C&L Inventory: Total number of notifiers: 77. • 1 has self-classified as Carc 1A • 2 have self-classified as	No CMR relevant information has been identified.

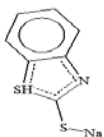| Identity	QSAR CMR[1] – Danish QSAR Database	QSAR predictions – other models[2]	Use[3], exposure & C&L	eChemPortal[4]
			Carc 1b and Mut 2 • 1 has self-classified as Carc 2 and Mut 2 • 73 have not self-classified as CMR	
1H-Naphth[2,3-f]isoindole-1,3,5,10(2H)-tetrone, 4,11-diamino-2-(3-methoxypropyl)- (C.I. Disperse Blue 60) CAS RN 12217-80-0 	CM Based on positive predictions in models for Ames, CA CHL, mouse lymphoma, mouse micronucleus, mouse SCE, and FDA RCA cancer call based on male rat and female rat.	<u>Cancer</u> Benigni-Bossa carcinogenicity (TOXTREE, v.1.0.0) = positive (good reliability) <u>Mutagenicity</u> CAESAR mutagenicity model (v.2.1.12) = positive (domain warning) T.E.S.T mutagenicity consensus model = positive Benigni-Bossa mutagenicity (TOXTREE, v.1.0.0) = positive (domain warning)	Use: Textile dye SPIN: Very narrow range of applications in DK (1-3) and no indication of exposure to humans (last record from 2008). Very narrow range of applications in NO and one or several uses indicate a very probable exposure to consumers (last record from 2010). Total tonnage in NO: confidential. ECHA C&L Inventory: Total number of notifiers: 92. No self-classifications for CMR.	No CMR relevant information has been identified.
Oxirane, 2,2'-[(1-methylethylidene)bis(4,1-cyclohexanediyloxymethylene)]bis- CAS RN 13410-58-7 	CMR Based on positive predictions in models for Ames, CA CHO, CA CHL, mouse lymphoma, Drosophila m. SLRL, mouse Comet, FDA RCA cancer call based on male rat, female rat,	<u>Cancer</u> No predictions within AD obtained <u>Mutagenicity</u> CAESAR mutagenicity model (v.2.1.12) = equivocal (domain warning)	Use: Derivative of BPA (Bisphenol A). Used in epoxy resins; food and beverage cans. SPIN: No records from DK. Very narrow range of applications in SE (1-3) (last record from 2010). One or several uses indicate a potential	No CMR relevant information has been identified.

Identity	QSAR CMR[1] – Danish QSAR Database	QSAR predictions – other models[2]	Use[3], exposure & C&L	eChemPortal[4]
	male mouse and female mouse.	SarPy model (v.1.0.6) = positive (domain warning) T.E.S.T mutagenicity consensus model = positive <u>Reproductive toxicity</u> CAESAR developmental toxicity model (v.2.1.6) = positive (domain warning) T.E.S.T developmental toxicity consensus model = negative	exposure to consumers and workers. Total tonnage in SE: confidential. ECHA C&L Inventory: Total number of notifiers: 51. No self-classifications for CMR.	
Oxirane, 2,2'-[1,4-cyclohexanediylbis(methyleneoxymethylene)]bis- CAS RN 14228-73-0	CMR Based on positive predictions in models for Ames, CA CHO, CA CHL, mouse lymphoma, Drosophila m. SLRL, mouse Comet, FDA RCA cancer call based on male mouse and female mouse.	<u>Cancer</u> No predictions within AD obtained <u>Mutagenicity</u> CAESAR mutagenicity model (v.2.1.12) = positive (good reliability) SarPy model (v.1.0.6) = positive (good reliability) T.E.S.T mutagenicity consensus model = positive Benigni-Bossa mutagenicity (TOXTREE, v.1.0.0) = positive (domain warning) <u>Reproductive toxicity</u> T.E.S.T developmental toxicity consensus model = negative	Use: Used in epoxy resins SPIN: Narrow range of applications in DK (4-10) (last record from 2010). One or several uses indicate a probable exposure to workers. Total tonnage in DK: 0.5 Tonnes (last record from 2010). An old record exists with 28 tonnes in DK in 2003. ECHA C&L Inventory: Total number of notifiers: 396. No self-classifications for CMR.	No CMR relevant information has been identified.
Propanamide, 2,3-dibromo-	C Based on positive predictions	No predictions within AD obtained	Use: Research chemical SPIN: No records from DK. Very	No CMR relevant information has been identified.

Identity	QSAR CMR[1] – Danish QSAR Database	QSAR predictions – other models[2]	Use[3], exposure & C&L	eChemPortal[4]
CAS RN 15102-42-8	in models for Ames, and FDA RCA cancer call based on male rat, female rat, male mouse and female mouse.		narrow range of applications in SE (1-3) (last record from 2010). One or several uses indicate a potential exposure to consumers and a probable exposure to workers. Total tonnage in SE: confidential. ECHA C&L Inventory: Total number of notifiers: 29. No self-classifications for CMR.	
Oxirane, 2,2'-[methylenebis(phenyleneoxymethylene)]bis- CAS RN 39817-09-9	CMR Based on positive predictions in models for Ames, CA CHO, CA CHL, mouse lymphoma, Drosophila m. SLRL, mouse SCE, mouse Comet, FDA RCA cancer call based on male rat, female rat, male mouse and female mouse.	Cancer Benigni-Bossa carcinogenicity (TOXTREE, v.1.0.0) = positive (domain warning) Mutagenicity CAESAR mutagenicity model (v.2.1.12) = positive (domain warning) SarPy model (v.1.0.6) = positive (domain warning) T.E.S.T mutagenicity consensus model = positive Benigni-Bossa mutagenicity (TOXTREE, v.1.0.0) = positive (domain warning) Reproductive toxicity T.E.S.T developmental toxicity consensus model = positive	Use: Derivative of BPF (Bisphenol F). Used in epoxy resins; food and beverage cans. SPIN: No records from DK. Narrow range of applications in SE (4-10) (last record from 2010). One or several uses indicate a very probable exposure to workers. Total tonnage in SE: <0.1 tonnes. An older record exists with 43 tonnes in DK in2003. ECHA C&L Inventory: Total number of notifiers: 81. No self-classifications for CMR.	No CMR relevant information has been identified.

Identity	QSAR CMR[1] – Danish QSAR Database	QSAR predictions – other models[2]	Use[3], exposure & C&L	eChemPortal[4]
1,4-Methanonaphthalen-6(2H)-one, octahydro-7-methyl- CAS RN 41724-19-0 	R Based on positive prediction in model for human teratogenicity.	CAESAR developmental toxicity model (v.2.1.6) = positive (good reliability) T.E.S.T developmental toxicity consensus model = negative	Use: Fragrance agent SPIN: Very narrow range of applications in DK (1-3) (latest record from 2010). One or several uses indicate a potential exposure to consumers and workers. Total tonnage in DK: confidential. ECHA C&L Inventory: Total number of notifiers: 900. No self-classifications for CMR.	No CMR relevant information has been identified.
Cyclopropanecarboxylic acid, 3-(2,2-dichloroethenyl)-2,2-dimethyl-, ethyl ester CAS RN 59609-49-3 	C Based on positive predictions in models for Ames, mouse lymphoma, FDA RCA cancer call based on male mouse and female mouse.	No predictions within AD obtained	Use: No information found SPIN: No records from DK. Very narrow range of applications in SE(1-3) (latest record from 2010). One or several uses indicate a probable exposure to workers and consumers. Total tonnage in SE: confidential. ECHA C&L Inventory: Total number of notifiers: 23. No self-classifications for CMR.	No CMR relevant information has been identified.

Identity	QSAR CMR[1] – Danish QSAR Database	QSAR predictions – other models[2]	Use[3], exposure & C&L	eChemPortal[4]
Oxirane, 2,2'-[[2-ethyl-2-[(oxiranylmethoxy)methyl]-1,3-propanediyl]bis(oxymethylene)]bis- CAS RN 3454-29-3 	CMR Based on positive predictions in models for Ames, CA CHO, CA CHL, mouse lymphoma, Drosophila m. SLRL mouse Comet, FDA RCA cancer call based on male mouse and female mouse.	Cancer CAESAR carcinogenicity model (v.2.1.8) = positive (domain warning) Mutagenicity CAESAR mutagenicity model (v.2.1.12) = positive (good reliability) SarPy model (v.1.0.6) = positive (good reliability) T.E.S.T mutagenicity consensus model = positive Reproductive toxicity CAESAR developmental toxicity model (v.2.1.6) = negative (domain warning) T.E.S.T developmental toxicity consensus model = positive	Use: Used in epoxy resins. SPIN: No records from DK. Narrow range of applications in SE & NO (4-10). One or several uses indicate a potential exposure to consumers and a very probable exposure to workers. Total tonnage: 1.1 tonnes in SE and 1 tonne in NO. ECHA C&L Inventory: Total number of notifiers: 155. No self-classifications for CMR.	No CMR relevant information has been identified.

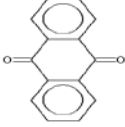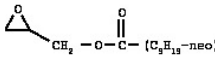# Annex 6 QSAR CMR predicted registered substances, illustrative cases

Identity	QSAR CMR[5] – Danish QSAR Database	QSAR predictions – other models[6]	Use[7] and C&L	REACH registration and eChemPortal[8]
Sodium 2-mercaptobenzothiazole (MBT Sodium salt) CAS RN 2492-26-4	C Based on positive predictions in models for CA CHO (part of training set), mouse lymphoma (part of training set), FDA RCA cancer call based on male rat (part of training set), female rat (part of training set), and female mouse (part of training set).	No predictions within AD obtained	Use: Corrosion inhibitor in automobile antifreeze solution, thermal stabilizer in silicon fluids, preservative in latex paint and on wood, and component of agricultural pesticides REACH registration: Tonnage: 10,000 – 100,000 Tonnes/year. Only Industrial use. Not self-classified for CMR.	REACH registration: four studies are cited. Two studies with mixed formulations are reported as negative (reliability 4 – not assignable). In addition, two studies are reported for a structural analogue. These are giving indications of some carcinogenic effects. ESIS: HPVC IARC: Possibly carcinogenic to humans (Group 2B)
9,10-Anthracenedione (Anthraquinone) CAS RN 84-65-1	C Based on positive predictions in models for Ames (part of training set), mouse lymphoma, and FDA RCA cancer call based on male rat (part of training set), female rat (part of training	CAESAR carcinogenicity model (v.2.1.8) = positive (good reliability) Benigni-Bossa carcinogenicity (TOXTREE, v.1.0.0) = positive (good reliability) (part of training set, experimental value = positive)	Use: Intermediate in the manufacture of dyes and pigments; bird repellent. REACH registration: Tonnage: 1,000 – 10,000 Tonnes/year. Industrial and	REACH registration: The NTP studies are cited in the registration dossier. The registrant concludes that the effects are not sufficient for classification as carcinogenic. ESIS: HPVC CPDB; negative cancer test in mice.

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[5] The underlying QSAR models for the resulting positive C, M or R call are listed. QSAR models in the CMR algorithms which are not mentioned may either have given negative predictions or the chemical may have been out of the applicability domain. No information is included about the manual evaluations of the predictions or information from other CMR relevant models and systems applied.
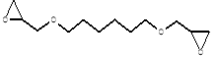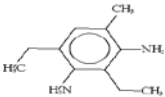
[6] Predictions generated in CMR models in CAESAR, TOXTREE, SarPy and T.E.S.T. Obtained predictions with reported low reliability or outside applicability domain are not reported.

[7] Quick Google searches and the US EPA ACTOR site primarily used to find information.

[8] The experimental results listed in this column are the non-comprehensive results of a quick look-up.

Identity	QSAR CMR[5] – Danish QSAR Database	QSAR predictions – other models[6]	Use[7] and C&L	REACH registration and eChemPortal[8]
	set), male mouse (part of training set) and female mouse (part of training set).		professional use. Not self-classified for CMR.	NTP; clear evidence of cancer in female rats and male/ female mouse, and some evidence in male rats. IARC: In prep.
Neodecanoic acid, oxiranylmethyl ester (selected analogs) (2,3-epoxypropyl neodecanoate) CAS RN 26761-45-5 	CR Based on positive predictions in models for Ames, mouse lymphoma, Drosophila m. SLRL, and FDA RCA cancer call based on male rat, female rat, male mouse and female mouse.	Cancer No predictions within AD obtained Mutagenicity CAESAR mutagenicity model (v.2.1.12) = equivocal (domain warning) (part of training set, experimental value = positive) SarPy model (v.1.0.6) = positive (domain warning (part of training set, experimental value = positive) T.E.S.T mutagenicity consensus model = negative Reproductive toxicity T.E.S.T developmental toxicity consensus model = positive	Use: A reactive diluent for epoxy resins in industrial coatings. REACH registration: Tonnage: 10,000 – 100,000 Tonnes/year. Industrial and professional use. Self-classified as MUTA 2 (CLP) (the registration dossier was updated with the MUTA 2 classification close to the publication date for this report).	REACH registration[9]: According to information from the registration dossier the substance is mutagenic in-vivo in the TGR assay in bone-marrow and liver tissue. Based on these results the substance is self classified as MUTA 2 (CLP). The data for the male germ cell mutant frequencies was incomplete at the time of registration. Negative in OECD 486 (UDS). No carcinogenicity studies are available and a testing proposal has been submitted for a 2-generation reproductive toxicity study and a pre-natal developmental toxicity study. ESIS: HPVC HPVIS: Weak positive in CA in vitro and positive in Ames, negative for DNA single strand damage with single dose in rats.

[9] When the substance was chosen as an illustrative case the registration dossier did not contain the MUTA2 self-classification, which appeared in a later update of the registration dossier. However, it was chosen to keep the substance in this table of illustrative QSAR cases.

Identity	QSAR CMR[5] – Danish QSAR Database	QSAR predictions – other models[6]	Use[7] and C&L	REACH registration and eChemPortal[8]
1,6-Hexanediol-diglycidyl ether CAS RN 16096-31-4	C Based on positive predictions in models for CA CHO, CA CHL, and FDA RCA cancer call based on female rat, male mouse and female mouse.	Cancer No predictions within AD obtained Mutagenicity CAESAR mutagenicity model (v.2.1.12) = positive (good reliability) SarPy model (v.1.0.6) = positive (good reliability) T.E.S.T mutagenicity consensus model = positive Benigni-Bossa mutagenicity (TOXTREE, v.1.0.0) =positive (domain warning)	Use: In applications such as adhesives, civil engineering projects, structural composites, marine and protective coatings, and potting and encapsulation of electronic components. REACH registration: Tonnage: 1,000 – 10,000 Tonnes/year. Industrial, professional and consumer use. Not self-classified for CMR.	REACH registration: positive in vitro in AMES. However, negative *in vivo* results in OECD TG 486 (UDS *in vivo*) and 474 (MN *in vivo*). No carcinogenicity studies are available. ESIS: LPVC eChemPortal: No CMR relevant information
Benzenediamine, ar,ar-diethyl-ar-methyl- (selected analogs) (Diethyl toluenediamine) CAS RN 68479-98-1	C Based on positive predictions in models for Ames, CA CHO, mouse lymphoma, and FDA RCA cancer call based on male mouse and female mouse.	Benigni-Bossa carcinogenicity (TOXTREE, v.1.0.0) = positive (domain warning)	Use: Chain extender for elastomeric polyurethanes and also a curing agent for epoxies. REACH registration: Tonnage: 1,000 – 10,000 Tonnes/year. Industrial and professional use. Not self-classified for CMR. Has a harmonized classification without CMR.	REACH registration: A carcinogenicity study (OECD TG 451) has been carried out. Some effects are described in the robust study summary such as a significant increase in hepatocellular adenomas in female rats, follicular cell adomas and follicular cell hyperplasia/follicular cysts in high dose females and thyroid follicular cell adenomas in male rats. However, according to the registrant: "*The neoplastic outcomes paralleled biologically significant decreases in body weight, and appeared to be secondary to excessive toxicity from exceeding the maximum tolerated dose of the test substance*".

Identity	QSAR CMR[5] – Danish QSAR Database	QSAR predictions – other models[6]	Use[7] and C&L	REACH registration and eChemPortal[8]
				ESIS: HPVC eChemPortal: No CMR relevant information

Use of QSAR to identify potential CMR substances of relevance under the REACH regulation
Substances classified as carcinogenic, mutagenic or toxic for reproduction (CMR) (>1 Ton) should have been registered under RECAH by December 2010. However, it is well-known that for low tonnage EU industrial substances the majority have few or no experimental CMR test data. QSAR models were applied to screen around 68,000 REACH pre-registered substances for CMR properties. The Nordic substance register database (SPIN) was used to identify not registered substances where human exposure is likely to be significant. The results can serve as an observation list for Industry and can be used by authorities for future priority setting for e.g. targeted experimental confirmatory testing and for the REACH registered substances for potential inclusion on the EU CORAP list.

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