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Can chemical structure predict reproductive toxicity?

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Abstract

Can chemical structure predict reproductive toxicity?

Structure-Activity Relationships (SARs), including Quantitative SARs, are applied to the hazard assessment of chemicals. This need is all the more urgent considering the proposed new EU policy on chemicals in REACH, which stresses the need for non-animal testing. DEREKfW and the TSCA Chemical Category List of the New Chemicals Program of the US-EPA were chosen to predict reproductive toxicity for REACH purposes. DEREKfW is a software program predicting the toxicological properties using the literature and expert-derived structural alerts, while the TSCA Chemical Category List is a document and is based on expert judgment and a category approach. We screened the performance of both models on recognizing substances that are classified for reproductive toxicity in the EU (based on experimental animal tests). As we limited our research to only reproductive positive examples the rate of false positives could not be assessed. DEREKfW partially fulfils the OECD principles for good (Q)SARs. DEREKfW and TSCA Chemical Category List did not recognize 90 and 77% of the substances classified for 'impaired fertility', and 81 and 82% of the substances classified for 'harm to the unborn child', respectively. Besides one mutual 'alert/category' DEREKfW and TSCA contain 7 'alerts' and 10 categories, respectively. As the alerts in DEREKfW (comprehensible and transparent tool) and categories in the TSCA Chemical Category List are highlighted in this research, they both can be used as additional expert judgment when assessing chemicals for reproductive toxicity. However, we conclude that these models cannot be the only method for screening chemicals for reproductive toxicity in the framework of REACH. Other models or testing strategies have to be used to assess reproductive toxicity of chemicals.

Key words: SAR, reproduction, DEREKfW, TSCA, Classification

Rapport in het kort

Kan chemische structuur reproductie toxiciteit voorspellen?

Structuur-activiteitsrelaties (SARs), inclusief kwantitatieve SARs worden gebruikt in de risicobeoordeling van stoffen. Deze noodzaak is des te meer urgent gezien het voorgestelde EU beleid voor stoffen, REACH, die de vermindering van dierproeven benadrukt. DERKfW en TSCA Chemische Categorieën Lijst zijn gekozen om reproductie toxiciteit voor REACH doeleinden te voorspellen. DEREKfW is een software programma dat toxicologische eigenschappen voorspelt gebruik makend van op literatuur en 'expert judgement' gebaseerde 'structural alerts', terwijl de TSCA Nieuwe Stoffen Programma Lijst van de US-EPA is gebaseerd op expert judgement en chemische categorieën. We hebben de twee modellen gescreened op het herkennen van stoffen die geclassificeerd zijn voor reprotoxiciteit in de EU (gebaseerd op experimentele dierstudies). De mate van vals positieven kon hierdoor niet bepaald worden. DEREKfW en de TSCA Chemische Categorieen Lijst herkenden 90 en 77% van de stoffen niet met een 'verminderde fertiliteit classificatie en 81 and 82% van de stoffen niet met een 'schade aan het ongeboren kind' classificatie, respectievelijk. Afgezien van één gezamenlijke 'alert' hebben DEREKfW (een helder model) and de TSCA chemische Categorieën Lijst nog 7 'alerts' en 10 categorieën, respectievelijk. Doordat de alerts in DEREKfW and TSCA Categorieën Lijst in dit onderzoek naar voren komen, kunnen deze gebruikt worden als additionele 'expert judgement'. We concluderen echter dat deze modellen niet de enige methode kunnen zijn voor het screenen van stoffen voor reproductie toxiciteit in het kader van REACH. Andere modellen en teststrategieën zijn nodig om reproductie toxiciteit van stoffen te beoordelen.

Trefwoorden: SAR, reproductie, DEREKfW, TSCA, Klassificatie

Preface

The authors thank Edith Laraway and Liz Covey-Crump of LHASA for critically reading the report and for the appropriate suggestions. The authors also like to thank LHASA for their permission presenting of the DEREKfW predictions of the EU reproductive classified chemicals. The authors also like to thank Betty Hakkert for her elucidating remarks.

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Summary

Structure-Activity Relationships (SARs), including Quantitative SARs, are applied to the assessment of chemicals. If the use of (Q)SARs here is to be enhanced, some questions will need to be answered concerning their validity. This need is all the more urgent considering the proposed new EU policy on chemicals in REACH, the new policy on chemicals, which stresses the need for non-animal testing (**R**egistration, **E**valuation and **A**uthorisation of **Ch**emicals).

Our aim was to investigate the validity of SAR tools for predicting reproductive toxicity. Two types of SAR models were chosen for the investigation, DEREKfW and the TSCA List of the New Chemicals Program. DEREKfW (**D**eductive **E**stimation of **R**isk from **E**xisting **K**nowledge **f**or **W**indows) is a software program to predict the toxicological properties using the literature and expert-derived structural alerts, while the TSCA (**T**oxic **S**ubstances **C**ontrol **A**ct) New Chemicals Program List of the US-EPA is based on expert judgment and a category approach. Both models are analysed and their predicting structure and content are described in detail in this report.

The compliance of DEREKfW with the so-called OECD (Q)SAR principles, a set of principles developed by the *Ad hoc* Expert Group on (Q)SARs, was investigated. A model which is intended to be used for regulatory purposes should fulfil the following criteria:

1) a model should be associated with a defined endpoint which it serves to predict;
2) take the form of an unambiguous and easily applicable algorithm for predicting a pharmacotoxicological endpoint;
3) ideally, have a clear mechanistic basis;
4) be accompanied by a definition of the domain of its applicability;
5) be associated with a measure of its goodness-of-fit (internal validation);
6) be assessed in terms of its predictive power by using data that were not used in the development of the model (external validation).

We screened the performance of the reproductive toxicity SARs in DEREKfW en the TSCA Chemical Category List on recognising substances classified for reprotoxicity within the scope of Directive 67/548/EC. This set of reprotoxic substances is listed, including all structural formulas, CAS numbers and classifications. As we limited our research to reproductive positive examples the rate of false positives could not be assessed.

Although OECD QSAR principles were not completely straight forward, it was concluded, that DEREKfW partially met these principles. For the 1st principle (defined endpoint) the endpoints were indeed established, and the details are available in the references of the alert description. However, the explanatory definitions of the endpoints were missing in the model and in the manual. The second principle (clear descriptors and hierarchy) is mostly fulfilled for the reproductive endpoint as the DEREKfW contains substituents specifying the requirements of the structural formula, which is true for most alerts for the reproductive endpoint. Principle 3 (clear mechanistic basis) is partly fulfilled, as structural alerts and corresponding references are available. The description of the mechanistic basis is given for most alerts, but at times insufficiently.
The 4th principle (defined domain of applicability) was fulfilled to a certain extent. The influence of other activating and deactivating substituents, not directly associated with the structural alert is lacking. The 5th principle (internal validation, training set available) was

considered to be fulfilled to a limited extent as the training set is limitedly available in the references and only a number of positive examples are underpinning the predictions. Principle six (external validation), explained as ‘model has been tested with data not used for developing the model’, is fulfilled, as published data on testing DEREKfW with sets of ‘external’ substances are available.

DEREKfW did not recognize 90% of the substances classified for ‘impaired fertility’ and 79% of the substances classified for ‘harm to the unborn child’. The TSCA Chemical Category List missed 77% and 82% of the cases, respectively. These values might still be too positive as the training set was partially unknown. Most chemicals were not detected by either method, due to the limited number of structural alerts available and the complex mechanisms of reproductive toxicity. Besides one mutual ‘alert/category’ DEREKfW and TSCA contain 7 ‘alerts’ and 10 categories, respectively. As the alerts in DEREKfW (comprehensible and transparent tool) and categories in the TSCA Chemical Category List are highlighted in this research, they both can be used as additional expert judgment when assessing chemicals for reproductive toxicity. However, we conclude that these models cannot be the only method for screening chemicals for reproductive toxicity in the framework of REACH. Other models or testing strategies have to be used to assess reproductive toxicity of chemicals.

At present, there is no complete collection of SARs for the reproductive endpoint. Reproductive toxicology is very complex and has several different and usually unknown mechanisms. Knowledge about this process is limited and SARs are difficult to define. An attempt to collect and describe the published structural alerts for the reproductive toxicity can be found in Hulzebos et al. (1999, 2001). It would be worthwhile to explore this area further. The predictions of TOPKAT and Multicase for the same performance set will be reported next year in co-operation with the Danish EPA.

Samenvatting

Structuur-activiteitsrelaties (SARs), inclusief kwantitatieve SARs worden gebruikt in de risicobeoordeling van stoffen. Als het gebruik van (Q)SAR toeneemt in de stoffen beoordeling is het nodig om vragen over hun validiteit aan te geven. Deze noodzaak is des te meer urgent gezien het voorgestelde EU beleid voor stoffen, REACH (**R**egistration, **E**valuation and **A**uthorisation of **Ch**emicals), die de vermindering van dierproeven benadrukt.

Ons doel is om de validiteit van SARs voor het voorspellen van reprotoxiciteit te bepalen. Voor dit onderzoek zijn twee modellen geselecteerd: DEREKfW and the TSCA Chemische Categorieën Lijst. DEREKfW (**D**eductive **E**stimation of **R**isk from **E**xisting **K**nowledge **f**or **W**indows) is een software programma dat gebruik maakt van op literatuur en 'expert judgment' gebaseerde 'structural alerts'. US Environmental Protection Agency (US-EPA ontwikkelt de TSCA (**T**oxic **S**ubstances **C**ontrol **A**ct) List voor het 'New Chemicals Program'. Het doel van de lijst is om stoffen te herkennen en te groeperen met gemeenschappelijke chemische en toxicologische eigenschappen. Beide modellen zijn geanalyseerd op opbouw en inhoud.

De overeenstemming met DEREKfW met de zogenoemde 'OECD (Q)SAR principles' is onderzocht. Deze principes betekenen dat (Q)SAR modellen:
1) een gedefinieerd eindpunt moeten voorspellen;
2) een ondubbelzinnige en eenvoudig toepasbare algoritme moeten hebben om de voorspelling te doen;
3) idealiter, een mechanistische basis moeten hebben;
4) het bereik van QSAR methode moeten aangeven;
5) geëvalueerd moeten zijn met een 'goodness-of-fit (interne validatie);
6) geëvalueerd moeten zijn in termen van voorspellingskracht door data te gebruiken die niet gebruikt zijn bij de ontwikkeling van het model (externe validatie).

We hebben de reprotoxische SARs in DEREKfW en the TSCA Chemische Categorieën Lijst gesreened op het herkennen van industriële stoffen die geclassificeerd zijn voor reprotoxiciteit in het kader van de Directive 67/548/EC. Aangezien we ons onderzoek beperkt hebben tot alleen positieve reprotoxische stoffen hebben getest kunnen we niets zeggen over het aantal vals positieven die de modellen geven.

DEREKfW vervult de OECD (Q)SAR 'principles' gedeeltelijk. Voor het eerste principe (gedefinieerd eindpunt), zijn de eindpunten inderdaad vastgesteld in de referenties, maar het eindpunt is verder niet beschreven in het handboek of het model. Het tweede principe (duidelijke descriptoren en hiërarchie) waarin de descriptoren gebruikt kunnen worden, is meestal wel vervuld, omdat DEREKfW substituenten beschrijft die aan de 'structural alert'gehecht mogen zijn. Het derde principe is voor een deel vervuld, omdat 'structural alerts' en referenties aanwezig zijn. Het mechanisme is beschreven voor het grootste deel van de alerts maar is soms onvoldoende Het vierde principe, dat het domein beschrijft van de (Q)SAR, is gedeeltelijk beschreven. De invloed van andere actieve substituenten, niet direct gehecht aan de 'structural alert' is niet gegeven. Principe vijf (interne validatie, training set beschikbaar) is beperkt aanwezig. De trainingset is als zodanig niet beschreven, maar is weergegeven in de genoemde referenties. Vaak zijn voorbeelden beschikbaar van stoffen met een vergelijkbare 'structural alert' die positief getest is in een experimentele dier studie. Aan

principe zes (externe validatie), uitgelegd als getest met een onafhankelijke test set is voldaan.
DEREKfW herkenden 90% van de stoffen niet met een ‘verminderde fertiliteit classificatie en 81% van de stoffen niet met een ‘schade aan het ongeboren kind’ classificatie. De TSCA Chemische Categorieën Lijst mist 77 en 82% van de stoffen met deze classificaties, respectievelijk. Deze getallen zijn mogelijk nog overschat aangezien de training set voor een deel niet bekend was. De meeste stoffen zijn niet herkend door beide methoden, door het beperkte aantal alert/categorieën en het complexe mechanisme van reproductie toxiciteit. Afgezien van één gezamenlijke ‘alert’ hebben DEREKfW and de TSCA chemische Categorieën Lijst nog 7 ‘alerts’ en 10 categorieën, respectievelijk. Doordat alerts in DEREKfW (een helder model) and TSCA Categorieën Lijst in dit onderzoek naar voren komen kunnen deze gebruikt worden als additionele ‘expert judgement’. We concluderen echter dat deze modellen niet de enige methode kunnen zijn voor het screenen van stoffen voor reproductie toxiciteit in het kader van REACH. Andere modellen en teststrategieën zijn nodig om reproductie toxiciteit van stoffen te beoordelen.

Momenteel is geen complete verzameling van SARs beschikbaar voor het reprotoxische eindpunt. Reproductie toxicologie is erg complex en kent vele verschillen en onbekende mechanismen. Kennis op dit gebied is beperkt en SARs zijn moeilijk te definiëren. Een aanzet om alle structural alerts voor reprotoxiciteit te verzamelen is uitgevoerd in Hulzebos et al. (2001, 1999). Deze aanzet zou verder uitgebouwd en onderbouwd kunnen worden. De voorspellingen met twee andere (Q)SAR modellen namelijk TOPKAT and Multicase voor dezelfde stoffen zullen volgend jaar gerapporteerd worden in samenwerking met de Deense EPA.

1. Introduction

1.1 Reproductive toxicity and REACH

In reproductive toxicity studies a very large number of laboratory animals are used. For example: a standard developmental toxicity test requires at least 160 animals, not to mention the pups of the first generation. Reproductive studies are very difficult to perform, labour intensive, time consuming and expensive.

A proposal for a new chemicals policy in the European Union called REACH (**R**egistration, **E**valuation and **A**uthorisation of **Ch**emicals) was recently published (EC, 2003c). The main principle is that all industrial chemical sold in quantities over one tonne each year have to be registered. If a substance is considered to be of concern (i.e. classified as a category 1 or 2 carcinogen, mutagen, as toxic to reproduction category 1 or 2, as substances that are (very) persistent, (very) bioaccumulative and toxic or as endocrine disrupters), it must be registered and evaluated or authorised. In practice this means that approximately 5,500 substances which are produced in large quantities or which are 'suspect' should be evaluated before 2008, and another 24,500 must comply with regulations by 2012 at the latest (TNO, 2002). There is very little existing data available for most of these substances. For chemicals with the highest production volume only for 14% have a base set available according to Directive 67/584EEC (EC, 1967) 65% have less than the base set and 21% have no data at all (EC 2003b). An estimated 2893 developmental toxicity studies and 2135 two-generation reproduction toxicity studies will have to be performed, which will cost in total € 852 million (EC, 2003b). Apart from the unacceptable large number of experimental animals needed and the financial burden, this will also exceed the capacity of the existing testing facilities.

Therefore, REACH encourages the use, as far as practicable, of non-animal test methods and the development of alternative methods (EC, 2001c). One of these alternatives is the application of (Quantitative) Structure-Activity Relationships. QSARs are simplified mathematical models of complex chemical-biological interactions. SARs are qualitative relationships in the form of structural alerts (fragments of chemical structure), that include molecular substructures or fragments related to the presence or absence of activity. These theoretical models can be used to predict the physicochemical, toxicological and pharmacological properties of molecules. (Q)SARs are already widely used by the pharmaceutical industry, but only to a limited extent for the investigation of other chemicals (mainly for assessing environmental exposure risks).

1.2 Objectives

In order to enhance the application of SARs and QSARs in risk assessment there is an urgent need for answering questions regarding the validity of the available QSARs. The aim of this report is to investigate the performance and validity of SAR-tools that can predict reproductive toxicity, according to the OECD principles (OECD, 2004b). Reproductive toxicity was chosen as an endpoint, as this is an important criterion for REACH and because it is a qualitative and not quantitative endpoint. It is also an important criterion in view of the potential savings in resources and in experimental animals.

For this investigation two major SAR-models were chosen: DEREKfW and the TSCA Chemical Categories List. DEREKfW (**D**eductive **E**stimation of **R**isk from **E**xisting **K**nowledge **f**or **W**indows) is a rule-based expert system, predicting the toxicological properties of chemicals based on an analysis of their molecular structure. (LHASA 2002). The TSCA Chemical Category List was developed within the scope of the US Environmental Protection Agency's (EPA's) New Chemicals Program: chemicals with shared chemical and toxicological properties were grouped into Chemical Categories (TSCA, 2002).
The approach taken consists of two elements:

1. Investigation into the compliance of DEREKfW with the so-called OECD (Q)SAR principles (OECD, 2004a,b). This investigation was performed in a general way, for all endpoints, and subsequently more in detail for the reproductive toxicity endpoint;
2. Comparing DEREKfW en the TSCA Chemical Category List predictions for reproductive toxicity with the EU reproductive classified chemicals of Directive 67/548/EC (referred to as ‘Annex I’ of this Directive).

1.3 Structure of the report

In the Chapter 2 the computer model DEREKfW and the TSCA Chemical Category List are generally described. In the same chapter the performance set of ‘Annex I’ substances together with the methods of performing research are presented. In the third chapter the evaluation of DEREKfW according to OECD (Q)SAR principles is performed and performance of DEREKfW and the TSCA Chemical Category List predictions concerning the reproductive toxicity are discussed. The last chapter presents our discussion and conclusions concerning validity of the DEREKfW model and the performance of both models for predicting the reproductive toxicity endpoint. This chapter also presents the conclusion about how DEREKfW fulfils the OECD (Q)SAR principles. General recommendations for the improvement of the validated models and proposal for further investigations are also included.

2. Methods

Chemicals classified in the EU for reproductive toxicity (2.1and 2.4) were run through the software programme DEREKfW (2.5) and manually through the TSCA Chemical Category List (2.6). The goal of this exercise is to relate the prediction of both models to EU classification (e.g. testicular effect predicted in the model in relation to reproductive toxicity in the EU classification (2.7 and 2.9). In addition, the OECD (Q)SAR principles were applied to DEREKfW (2.8).

2.1 Annex I, Annex VI and TGD

In this chapter several sources come forward, which require some explanation and comments. It concerns documents used in the EU for the risk evaluation of new and existing chemical substances. The background for all these documents is the Dangerous Substances Directive (EC, 1967).

The Council Directive on Dangerous Substances specifies the hazard classification, packaging and labelling requirements for dangerous substances supplied in the European Union. The technical content of the Directive is contained in a number of Annexes. Two of them are used in this report:

- Annex I of Directive 67/548/EEC contains a list of harmonised classifications and labelling for substances or groups of substances, which are legally binding within the EU. The list is regularly updated through Adaptations to Technical Progress (e.g. 28th ATP) (EC, 2001a). Revised and new classifications inserted to the list are proposed by DG ENV and agreed by a Member State vote.
  The DG ENV proposal is based on advice from the Commission Working Group on Classification and Labelling with participation of experts from the Member States. Their meetings are prepared, chaired and followed-up by the ECB (EC, 2001). Annex I at present contains approximately 2550 existing and 700 new substance entries (EC, 2001a, 2004b).
- Annex VI Directive 67/548/EEC; how to classify a substance not yet present in Annex I (EC, 2004b). Usually it is referred to as the Classification and Labelling guide. If a dangerous substance not yet included in Annex I is put on the market (as a pure product or contained in a preparation), manufacturers/ importers/distributors have to self-classify the substance according to the criteria in Annex VI. Criteria for classification on the basis of the intrinsic (physical-chemical, toxicological and ecotoxicological) properties of a substance are described for each R(risk) phrase. Depending on the risk phrase, a safety (S) advice phrase may be required. Safety phrases give advice on how to handle a dangerous chemical. (EC, 2001b)

Besides the Dangerous Substances Directive (67/548/EEC), the EU Technical Guidance Document (EC, 2003a) is used. This document supports legislation on risk assessment for human health and environment of new, existing and biocidal chemical substances. It contains information on the required tests and test strategies, calculation of emissions, fate, and consumer/worker exposure. In addition some QSAR estimations are included.

2.2 Definitions and terminology for the assessment of the model outcome

2.2.1 Definitions

Some terms used in the literature and used in the present report are explained in this present paragraph.

Descriptor - A word, phrase, or alphanumeric character used to identify an item in an information storage and retrieval system. (American Heritage® Dictionary, 2004)
Domain of applicability for the SAR, the range of physicochemical properties or chemical classes of chemicals or certain substituents for which the SAR is applicable (OECD, 2004a).
Assessment endpoint - An explicit expression of a toxic response to a substance that is used as the basis of a health evaluation. In this report 'reproductive toxicity' is an assessment endpoint (OECD, 2003a).
MDL molfile – A molecular structure format file. The MOL file format is used to encode chemical structures, substructures and conformations as text-based connection tables (Van de Waterbeemd et al., 1997).
SAR – Structure Activity Relationship is an explicit description of the substructure (structural fragment or structural alert), including an explicit identification of its substituents, that underline the reactivity of the molecule (OECD, 2004b).
SMILES notation - (**S**implified **M**olecular **I**nput **L**ine **E**ntry **S**pecification). A simple, concise and rather readable molecular structure specification format (Weininger, 1988).
Specific endpoint – Some assessment endpoints may be expressed in several ways. For example, the assessment endpoint reproductive toxicity may be expressed as impaired fertility or developmental toxicity.
Structural fragment or Structural alert – Part of a chemical structure, which may be associated with a certain (toxicological) action. Together with the identified substituents, the structural fragment (structural alert) forms the SAR.
Toxophore – segments of molecule, which are associated with a specific activity (LHASA, 2002).

2.2.2 Terminology for the assessment of the model outcome

The criteria used for judging the trustworthiness of the SARs tested in this report are derived from the US ICCVAM (Interagency Coordinating Committee on the Validation of Alternative Methods) (ICCVAM, 2002). ICCVAM, as is ECVAM in Europe, is responsible, among others, for the validation of *in vitro* methods for toxicological endpoints. For this reason the *in vitro* test results of evaluated tests are compared with *in vivo* test results. Also other frameworks, which are working in this field, have established similar definitions (ECETOC, 2002 and ECVAM, 2002). In the present study we have compared the SAR predictions with the results of reproductive toxicity tests. The following terminology was used (ICCVAM, 2002):

- **Sensitivity** is defined as the proportion of all positive chemicals that are correctly classified as positive in a test.
- **Specificity** is defined as the proportion of all negative chemicals that are correctly classified as negative in a test.
- **Accuracy** (concordance) is defined as the proportion of correct outcomes of a method.
- **False positive rate** is defined as the proportion of all negative chemicals that are falsely identified as positive.

- **False negative rate** is defined as the proportion of all positive chemicals that are falsely identified as negative.

2.3 The test set: Annex I substances

The term 'reproductive toxicity' is used in the Technical Guidance Document of the EU to describe the adverse effects induced (by a substance) on any aspect of mammalian reproduction. It covers all phases of the reproductive cycle, including impairment of male and female reproductive function, capacity and the induction of non-heritable adverse effects in the progeny e.g. death, growth retardation, structural and functional effects (EC, 2003). According to the Classification and Labelling Guide, substances and preparations which are toxic for reproduction are defined as substances and preparations which, after inhalation, ingestion or skin penetration, may produce or increase the incidence of non-heritable adverse effects in the progeny and/or an impairment of male or female reproductive functions or capacity.

Reprotoxic chemicals, as described above, were for the purpose of this performance obtained from two sources. One of them was Annex I of the 28th adaptation of Directive 67/548/EEC (EC, 2001 a) as this already has official legal status. The second source was the working database of substances classified as agreed in the Commission Working Group of Classification and Labelling of July 2003, proposal for 29th ATP. This list of labelled substances has no official legal status yet, discussion still has to take place (EC, 2004b). The list is expected to be made officially within one year). Both databases are available on the internet at http://ecb.jrc.it/classification-labelling/. The two databases were chosen for the following reasons:

- all substances presented were evaluated according to one set of criteria, by the same experts;
- the data are not confidential;
- information on all substances is available at the ECB website (CAS number, chemical name and complete classification).

For chemicals which were included in both databases, the classification from the working database was used, because it also included additional, recently proposed classification. An overview of all chosen substances (108), including CAS numbers and classification for reprotoxic properties, is presented in Appendix 1.

2.4 Classification and labelling for reproductive toxicity

As mentioned previously (definition given in paragraph 2.3), reproductive toxicity may be divided in two parts:

Effects on male or female fertility includes adverse effects on libido, sexual behaviour, any aspects of spermatogenesis or oögenesis, hormonal activity and physiological response which could interfere with the capacity to fertilise, fertilisation itself or the developing ovum up to and including implantation (EC, 2001b). The effects are described by the risk phrases R60 (May impair fertility) and R62 (Possible risk of impaired fertility). The difference between these phrases is significant: R60 is assigned to substances which are known to impair fertility in human or should be regarded as if they impair human fertility; R62 is assigned to substances which cause concern for human fertility. Classification with R60 is more severe and based on more convincing evidence than classification with R62. For the detailed explanation see Table 1.

Developmental toxicity is defined in its broadest sense as any effect interfering with normal development, both before and after birth and includes embryotoxic/fetotoxic effects e.g. reduced body weight, growth and developmental retardation, organ toxicity, death, abortion, structural defects (teratogenic defects), functional defects, peri-postnatal defects, and impaired postnatal mental or physical development up to and including pubertal development (EC, 2001b). The effects are expressed/described by the risk phrase R61 (May cause harm to the unborn child) and R63 (Possible risk of harm to the unborn child.). Difference between these phrases is significant: R61 is assigned to substances which are known to cause

Table 1: R-phrases and explanation (EC, 2001 b).

R-sentence	Explanation
R60 (May impair fertility), (category 1 and 2)	Substances known to impair fertility in humans. There is sufficient evidence to establish a casual relationship between human exposure to the substance and impaired fertility. **Or** Substances which should be regarded as if they impair fertility in humans. There is sufficient evidence in animal studies of impaired fertility in the absence of toxic effects, or, evidence of impaired fertility occurring at around the same dose levels as other toxic effects but which is not a secondary non-specific consequence of the other toxic effects.
R62 (Possible risk of impaired fertility) (category 3)	Substances which cause concern for human fertility. Generally this conclusion is based on results in appropriate animal studies which provide sufficient evidence to cause a strong suspicion of impaired fertility in the absence of toxic effects, or, evidence of impaired fertility occurring at around the same dose levels as other toxic effects but which is not a secondary non-specific consequence of the other toxic effects, but where the evidence is insufficient to label substance with R60.
R61 (May cause harm to the unborn child), (category 1 and 2)	Substances known to cause developmental toxicity in humans. There is sufficient evidence to establish a casual relationship between human exposure to the substance and the subsequent developmental toxic effect in the progeny. **Or** Substances which should be regarded as if cause developmental toxicity to humans. There is sufficient evidence to prove a strong presumption that human exposure to the substance may result in developmental toxicity, generally on the basis of clear results in appropriate animal studies where effects have been observed in the absence of signs of marked maternal toxicity, or at around the same dose levels as other toxic effects but which is not a secondary non-specific consequence of the other toxic effects.
R63 (Possible risk of harm to the unborn child.) (category3)	Substances which cause concern for humans owing to possible developmental toxic effects. Generally this conclusion is based on results in appropriate animal studies which provide sufficient evidence to cause a strong suspicion of developmental toxicity in the absence of signs of marked maternal toxicity, or at around the same dose levels as other toxic effects but which is not a secondary non-specific consequence of the other toxic effects, but where the evidence is insufficient to label substance with R61.
R64 (May cause harm to breastfed babies)	Substances which are not classified as toxic to reproduction but which cause concern due to toxicity when transferred to the baby during the period of lactation. This R-phrase may also be appropriate for substances which affect the quantity or quality of the milk.

developmental toxicity in human or should be regarded as if they cause developmental toxicity in humans; R63 is assigned to substances which cause concern for humans owing to possible developmental toxic effects. Classification with R61 is more severe and is based on

a greater amount of evidence than the classification with R63. For a detailed explanation see Table 1.In addition, another endpoint for the reproductive toxicity was taken into account in this investigation. **Effects during lactation** concern the toxic effect on offspring resulting only from exposure via breast milk, or the effect on quality and quantity of milk. These effects are described in the risk phrase R64 (May cause harm to breastfed babies). For a detailed explanation see Table 1.
Summarising, reprotoxic chemicals were extracted from Annex I of the 28th adaptation of Directive 67/548/EEC (EC, 2001a) and the working database of substances classified as agreed in the Commission Working Group of Classification and Labelling, proposal for 29th ATP using the R-sentences: R60, R61, R62, R63 and R64.

2.5 DEREKfW – knowledge based system

2.5.1 General information on DEREKfW

DEREKfW (**D**eductive **E**stimation of **R**isk from **E**xisting **K**nowledge) is a rule-based expert system for predicting the toxicological properties of chemicals based on an analysis of their molecular structure (LHASA, 2002). The predictions are based on the following criteria:

- Structural alerts. The term structural alert refers to one or a combination of structural features in a molecule and gives a signal that a particular toxic effect may occur;
- Species;
- Toxicity data;
- Toxicological endpoint;
- Physico-chemical properties (e.g. log Kp for skin permeability and molecular weight).

The predictions are formed based on the above mentioned criteria and are used to form the so-called reasoning rule. Reasoning rules have the following formula (DEREKfW user guide, 2002):

If [Grounds] is [Threshold] then [Proposition] is [Force].

- *Grounds* is the evidence to be considered by the reasoning rule (for example SAR for certain toxicological endpoint);
- *Threshold* is the level above which the grounds must be for the proposition to be assigned the force (for example cut-off values or limitations considering the structure);
- *Proposition* is the outcome of the reasoning rule (for example a chemical is considered to be developmental toxicant);
- *Force* is the likelihood of the reasoning rule outcome (for example ‘plausible’, see section 1.5.3 for further explanation of likelihood levels).

As shown in figure 1, a reasoning rule leads to a conclusion which, sometimes in combination with other rules, leads to a toxicity prediction and the likelihood thereof.
All outcomes are peer reviewed by expert toxicologists and are supported by literature references (Greene et al., 1999).

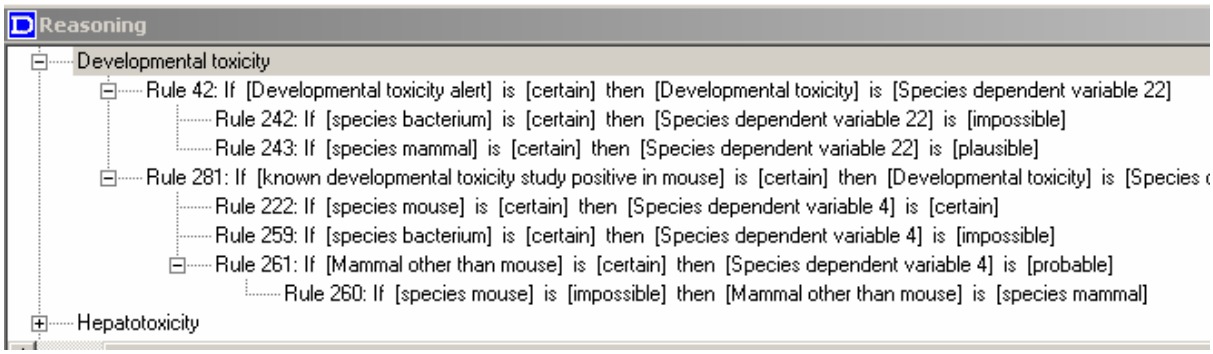

Figure 1: Example of reasoning rules for developmental toxicity.

2.5.2 Predicting part and Editor part

DEREKfW is divided into two main parts:

Part 1: Prediction of the toxicological properties of a chemical.

- Most endpoints are directly associated with a structural fragment, if a toxophore has been detected in the examined molecule. In other words a toxophore (SAR) forms *Grounds* for the reasoning rule. Figure 2 presents an example of the SAR based prediction for developmental toxicity, with a likelihood of 'plausible' (see 2.5.3). Structural fragments including domains, comments and references are always given for this type of prediction. As one can see on the Figure 2, examples in this case are not available.

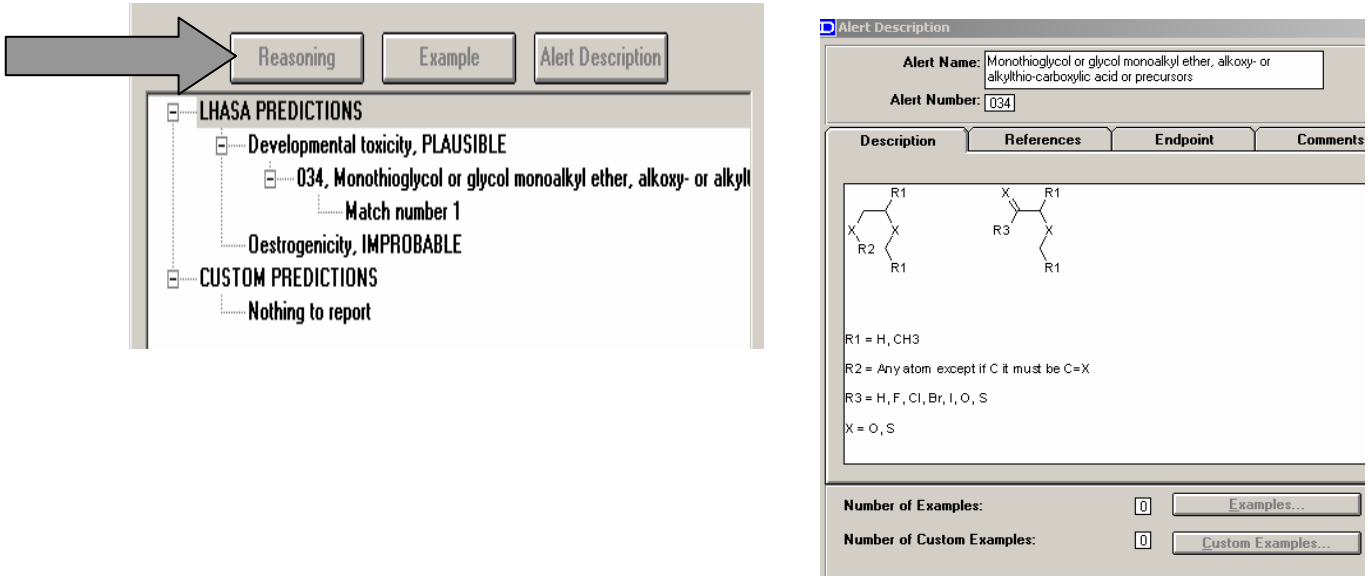

Figure 2: Example a DEREKfW prediction for developmental toxicity, based on structural fragment.

- Some endpoints are predicted by the reasoning engine and are associated with a reasoning rule, but not directly associated with the presence of a structural alert within the examined molecule. Figure 3 presents an example of the reasoning rule based prediction. In this case a partitioning rate for dermal absorption (log Kp) forms *Grounds* for the reasoning rule. No references are given. The reasoning for this prediction was that the log Kp, estimated by DEREKfW for the tested substance was within the range of required domain.

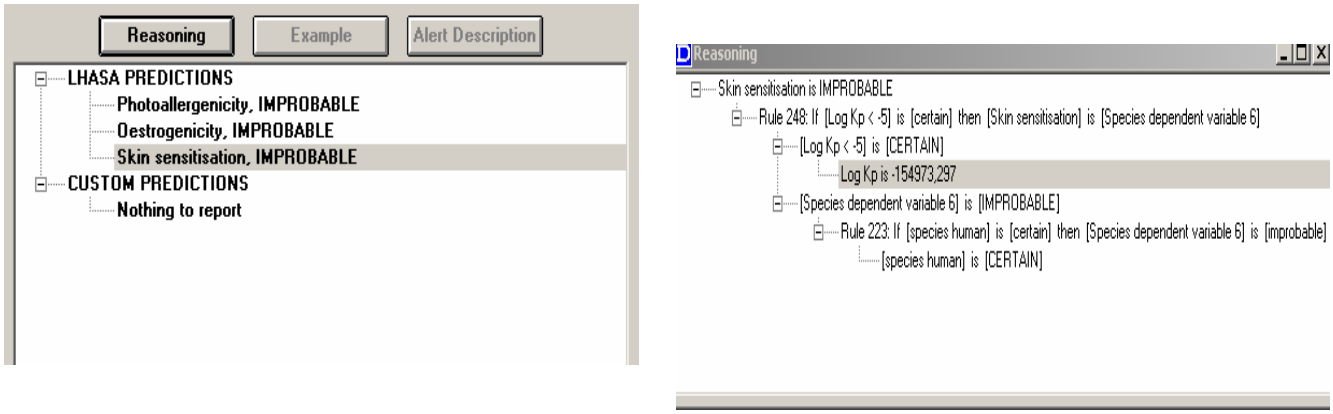

Figure 3: Example of DEREKfW prediction for skin sensitisation, based on the reasoning rule.

Part 2: Editor part. Here all endpoints and connected structural alerts are listed. If a known positive substance is incorrectly predicted, the researcher can check in the editor part, if a certain structural alert is present for the investigated endpoint. In addition, there are tools for users to add their own in-house alerts or reasoning rules.

The program covers the following toxicological endpoints: carcinogenicity, mutagenicity, skin sensitisation, teratogenicity, irritation, and respiratory sensitisation. Definitions of endpoints are not available in the model itself or in the manual. For the complete overview of the endpoints see Appendix 2.
It is possible to choose the species for which predictions are required e.g. humans, mammals (rat, mouse, guinea pig, hamster and primate) and bacteria. The chemical structures can be imported into DEREKfW via its automatic link to ISIS/Draw (computer model for drawing chemical structures) or by importing MDL Molfiles.

2.5.3 Likelihood levels in DEREKfW

When a structural alert or a match for a reasoning rule is found by DEREKfW in a molecule, the expected endpoint is indicated. The reliability of these predictions is presented in the form of one of eight levels of likelihood (e.g. see Figure 2 and 3). They are listed in the table below (LHASA, 2002).

Predictions associated with a structural alert are more transparent than predictions based on other grounds (Kp, molecular weight). In the first case references, examples and domain are available. In the second case only a cut-off value or domain is available, without references or further explanation. Note: Version 8.0 does contain references for some of these rules (Personal communication with LHASA).

Table 2: Levels of likelihood from DEREKfW and their definition.

Levels of likelihood	Definition
Certain	There is proof that the proposition is true
Probable	There is at least one strong argument that the proposition is true and there are no arguments against it
Plausible	The weight of evidence supports the proposition
Equivocal	There is an equal weight of evidence for and against the proposition
Doubted	The weight of evidence opposes the proposition
Improbable	There is at least one strong argument that the proposition is false and there are no arguments that it is true
Open	There is no evidence that supports or opposes the proposition
Contradicted	There is proof both that the proposition is true and that it is false

2.5.4 DEREKfW alerts for reproductive toxicity

There are 9 structural alerts included in DEREKfW for the reproductive toxicity endpoints. DEREKfW contains 3 alerts for developmental toxicity, 5 alerts for teratogenicity, 1 alert for testicular toxicity:

1. Polyalkyl urea: developmental toxicity, teratogenicity in rat foetus;
2. Monothioglycol or glycol monoalkyl ether, alkoxy- or alkylthio-carboxylic acid or precursors: developmental toxicity, teratogenicity/foetotoxicity;
3. Benzidine-based bisazo compound: developmental testicular toxicity (testis weight and enumeration of atrophic tubules);
4. Thalidomide-type compound: teratogenicity;
5. Short chain carboxylic acid or precursor: teratogenicity;
6. Pyrroline ester, pyrroline N-oxide ester, pyrrole ester or pyrrole alcohol: teratogenicity;
7. Triazole antifungal analogue: teratogenicity;
8. Retinoid or analogue: teratogenicity;
9. Monothioglycol or glycol monoalkyl ether, alkoxy- or alkylthio-carboxylic acid or precursors: testicular toxicity, testicular atrophy.

2.6 TSCA Chemical Categories List

The US Environmental Protection Agency (EPA) New Chemicals Program was established to help manage the potential risk from new chemicals. In 1987, notified chemicals with shared chemical and toxicological properties were grouped into categories, the so called Chemical Categories (TSCA, 2002). Currently, there are a total of 45 categories, listed in the TSCA report. A category statement contains:

- description of the molecular structure;
- boundary conditions such as molecular weight, equivalent weight, the log of the octanol/water partition coefficient (log P) and water solubility;
- standardised hazard and fate tests to address concerns for the category.

TSCA chemical category for reproductive toxicity

The following Categories are connected to reproductive toxicity:

1. Acrylamides: reproductive and developmental toxicants. There is no concern for chemicals with a MW of > 5000 and there is concern if MW is < 1000. The chemicals with a MW between 1000 and 5000 are assessed on a case by case basis;

2. Anhydrides, Carboxylic Acid: concern for potential developmental or reproductive toxicity based on data for maleic, succinic, and phthalic anhydrides;
3. Dianilines: potential retinotoxic agents by analogy to 4,4'-methylenedianiline, 4,4'-oxydianiline, and the diaminodiphenyl alkane drugs are also potential reproductive and systemic toxicants by analogy to 4,4'-methylenedianiline;
4. Benzotriazole-hindered phenols: Reproductive toxicity, including atrophy of the seminal vesicles, significant reduction in absolute and relative testes weight, significant reduction in absolute and relative prostate weight, and abnormal spermatogenesis. Boundaries cannot be given;
5. Boron compounds: reproductive toxicity (i.e., sterility in males and females, and testicular atrophy in males). Nothing is mentioned on boundaries;
6. Epoxides: reproductive effects. Epoxides with a MW > 1000 are not expected to cause concern;
7. Ethylene Glycol Ethers: Short-chain ethylene glycol ethers are developmental and reproductive toxicants. No concern is expected if the alkylchain is > C7;
8. Hindered Amines: toxic to male reproductive system;
9. Nickel Compounds: fetotoxicity. Ni^{2+} needs to be released for the effect;
10. Triarylmethane Pigments/Dyes with non-solubilizing Groups: developmental and reproductive toxicity. Dyes with soluble groups are not expected to be of concern, neither are insoluble pigments/dyes (1 ppb);
11. Vinyl Esters: Reproductive toxicity.

In the list boundaries are given whether the alerts can be used for prediction, e.g. molecular weight.

2.7 DEREKfW – Performance procedure

2.7.1 Technical details of testing DEREKfW

A special step-by-step procedure was introduced to avoid errors in the sketching of the structures of the chemicals. The SMILES notation of each chemical was produced from the CAS-number by EPIwin V3.10. The SMILES notation was copied into ACD/Chem Sketch Freeware v5.11. Chem Sketch (a computer drawing program for chemical structures) generated a structure from the smiles notation. A MDL molfile was exported from Chem Sketch and imported into DEREKfW 6.0 (of 2002/3, the last updated version released in 2004 is version 8.1). A prediction was considered positive if an alert was found by DEREKfW for testicular or developmental toxicity or teratogenicity. All inorganic substances were left out as no alerts for reproduction toxicity for this group of substances are present in DEREKfW. Some other substances were not used because only the name of the class was given or because the structural formula could not be reproduced. Overall, 108 substances were tested (See Appendix 1).

2.7.2 Comparison DEREKfW versus Annex VI definitions

The following reprotoxic endpoints are predicted in DEREKfW:

1. Developmental toxicity
2. Teratogenicity
3. Testicular toxicity

These endpoints are not further specified or defined in DEREKfW. It appeared that the terms used in the Annex VI classification guideline (EC, 2001b) and in DEREKfW are different.

Therefore, in this paragraph terms used in DEREKfW and the Annex VI definitions are compared and brought in line.

The DEREKfW endpoint **testicular toxicity** falls within the criteria for fertility (R60 and R62). However, the Annex VI criteria for fertility also include effects on females and other effects that could influence the fertility such as libido (EC, 2001b). For the purpose of this performance, all substances which were recognised by DEREKfW as testicular toxicants were regarded as impairing the fertility and comparable with R60/62.

The DEREKfW endpoints **teratogenicity and developmental toxicity** fall within the Annex VI criteria for developmental toxicity (R61 and R63). For the purpose of this performance, all substances which were recognised by DEREKfW as teratogenic or developmental toxicants were regarded to be comparable with this labelling.

No endpoint comparable to effects on lactation (R64) is available in DEREKfW. Therefore, no comparison with DEREKfW could be made.

Table 3 Comparison of classification from Annex 1 (EC 2001 a) and DEREKfW.

Classification Annex 1	Terms used in DEREKfW
R60 (May impair fertility) R62 (Possible risk of impaired fertility)	Testicular toxicity
R61 (May cause harm to the unborn child) R63 (Possible risk of harm to the unborn child.)	Teratogenicity Developmental toxicity

2.7.3 Interpretation of DEREKfW predictions

For all chemicals recognised by DEREKfW as having a structural alert for reproductive toxicity, prediction was given a level of likelihood ‘plausible’. Information on the background of the predictions is given in rules. The reliability was judged by careful examination of the related rule, references and example compounds. We considered all ‘plausible’ predictions as positive, and therefore accepted them as a positive prediction.

2.8 OECD (Q)SAR principles for (Q)SAR computer models

In March 2002 in Setubal (Portugal) a workshop was held on the use of (quantitative) structure activity relationships for regulatory purposes, as one of the components of the chemicals safety assessment (ECETOC, 2002). Any model used for regulatory purposes should be scientifically valid, appropriate for the purpose intended, reliable and accepted by decision-makers. To allow screening for the usefulness of existing models, the principles are developed by the *Ad Hoc* Expert Group on (Q)SARs and can found below:

1) a model should be associated with a defined endpoint which it serves to predict;
2) take the form of an unambiguous and easily applicable algorithm for predicting a (pharmaco)- toxicological endpoint;
3) a mechanistic interpretation, if possible
4) be accompanied by a definition of the domain of its applicability
5) be associated with a measure of its goodness-of-fit (internal validation);
6) be assessed in terms of its predictive power by using data that were not used in the development of the model (external validation).

A more recent document is has become available late 2004 in which the order of the principles is changed (a mechanistic interpretation is the last principle) and principle five and

six are combined (OECD, 2004b). These OECD (Q)SAR principles are applied to DEREKfW.

2.9 The TSCA Chemical Category List, performance procedure

Additionally to testing DEREKfW we tried to predict reprotoxic substance from Annex I using the TSCA Chemical Category List (TSCA, 2002). The structural formula of every Annex I chemical was compared with the structural alert and explanation given in the Chemical Category document. Cut-off values for the chemical structure and molecular weight were also taken into account. If a compound could be classified as a member of a TSCA Chemical Category, and the Annex I classification and labelling was comparable to the effects predicted for a category, we considered the prediction as positive. The terms used in the TSCA Chemical Category List were related to the Annex I classification and labelling risk sentences as follows:

Table 4: Comparison of classification from Annex 1 (EC 2001 a) and the TSCA Chemical Category List.

Classification Annex 1	Terms used in TSCA Chemical Category List
R60 (May impair fertility) R62 (Possible risk of impaired fertility)	Toxic to male reproductive system, Sterility in males and females, Testicular atrophy in males, Reproductive toxicity
R61 (May cause harm to the unborn child) R63 (Possible risk of harm to the unborn child.)	Fetotoxicity, Reproductive toxicity, Developmental toxicity,

The term ‘reproductive toxicity’ was understood as both impairing fertility and harmful to the unborn child, if no further specification was given in the TSCA Chemical Category List. In addition, the authors of the report assumed that when TSCA Chemical Category List stated that chemicals are possibly reproductive toxic that the NOAEL for reproductive toxicity is below 1000 mg/kg bw, as this is the maximum dose that need to be dosed according to OECD reproductive toxicity guidelines.

3. Results

3.1 DEREKfW versus OECD (Q)SAR principles

In this chapter the explanation of and comments to the OECD (Q)SAR principles are presented as they were in their draft form, together with the results of the DEREKfW evaluation using these criteria. A preliminary evaluation of DEREKfW was performed for all endpoints. Subsequently the endpoint reproductive toxicity was evaluated in a more detailed manner.

Setubal principle 1: A model should be associated with a defined endpoint which it serves to predict. A well defined (eco)toxicological endpoint (for example developmental toxicity, sensitization, irritation and corrosivity) should be present, which has clear relevance for defined purpose. In this case the purpose is the preliminary screening of the substances in order to select reprotoxic substances. The background information (i.e. experimental conditions and conditions of the performed tests) should be available (OECD, 2004b)
Comments concern Setubal principle 1:
General comments: This model contains 36 endpoints (see Appendix 2 for the overview table). Definitions of these endpoints are not available in DEREKfW and need to be retrieved from the references given together with the predictions. These toxicological endpoints are based on different numbers of structural alerts, varying from one to 77. Only a few endpoints are based on a significant number of structural alerts:

- Mutagenicity: 77 structural alerts;
- Skin sensitisation: 61 structural alerts;
- Carcinogenicity: 46 structural alerts;
- Skin irritation: 25 structural alerts;
- Eye irritation: 29 structural alerts;
- Respiratory track irritation: 19 structural alerts;
- Thyroid toxicity: 14 structural alerts;
- Respiratory sensitisation: 13 structural alerts.

For the other toxicological endpoints less than 10 structural alerts are given. For 13 endpoints only one structural alert is present.
Specific comments for reproductive toxicity. For the general endpoint reproductive toxicity there are three more specific endpoints available: developmental toxicity (3 structural alerts), teratogenicity (5 structural alerts) and testicular toxicity (1 structural alert). For examples and number of available references see table 5.

Table 5: Reproductive toxicity in DEREKfW.

Endpoint	Structural alert (alert description)	Number of references	Examples of active substances
Developmental toxicity	Polyalkyl urea	3	N, N'-dimethylurea, N,N,N'-trimethylurea and tetramethylurea
	Monothioglycol or glycol monoalkyl ether, alkoxy- or alkylthio-carboxylic acid or precursors	1	No examples
	Benzidine-based bisazo compound	1	Chlorazol black E Congo red Diamine blue
Teratogenicity	Thalidomide-type compound	3	Thalidomide
	Short chain carboxylic acid or precursor	6	No examples.
	Pyrroline ester, pyrroline N-oxide ester, pyrrole ester or pyrrole alcohol	5	No examples
	Triazole antifungal analogue	1	No examples
	Retinoid or analogue	4	No examples
Testicular toxicity	Monothioglycol or glycol monoalkyl ether, alkoxy- or alkylthio-carboxylic acid or precursors	1	No examples

Conclusion DEREKfW versus Setubal 1 principle: There are several toxicological endpoints available in DEREKfW. However, definitions of these endpoints are given only in the references given with predictions, they are not available in the manual or programme. The number of the structure alerts varies between endpoints from one to 77. Titles of references containing background information are always available, but examples of active substances are missing in many cases. For the general endpoint reproductive toxicity only a limited amount of structural alerts is available.

Additional conclusion: Authors of this paper noted, that it was rather difficult to make a clear distinction between the Setubal criteria 2 and 4.
Setubal principle 2: A model should take the form of an unambiguous and easily applicable algorithm for predicting a pharmaco-toxicological endpoint. (...) Individual structural alerts should not be considered for validation in isolation, but an integrated approach should be taken assessing all the rules at the same time in the context of their hierarchy. (OECD).
Setubal principle 4: A model should be accompanied by a definition of the domain of its applicability. (...) In the case of a SAR, information should be given if the substructure associated with any inclusion and/or exclusion rules on its applicability to groups of chemicals.
In both cases it might be understood, that also other substituents, next to the toxophore itself, should be taken into account. A distinction may be made between substituents accompanying the toxophore directly and other active substituents present in the molecule that may influence the toxicity. Setubal principle 2 and 4 show some overlap considering SAR principles Setubal 2 requires the description of the structural alerts including the substructural environment. This is closely related to Setubal principle 4, for in which also need to be described which atoms related to the alert belong to the domain of the alert and which do not.

Setubal principle 2: A model should take the form of an unambiguous and easily applicable algorithm for predicting a pharmacotoxicological endpoint. An explicit description of the substructure, including an explicit identification of its substituents should be present. (OECD, 2004).
Comments concerning Setubal principle 2:
General comments: In DEREKfW the relevant part of the molecule, being a structural alert valid for a certain specific endpoint, is marked red. If a structural alert is found by DEREKfW in a molecule and the domain requirements are met, the expected endpoint is indicated. Indications whether the substructure is associated with any inclusion and/or exclusion rules on its applicability to groups of chemicals are usually available. Cut-off values (for example, chain length and certain substituents) for the structural alerts are taken into account in most cases.

Specific comments for reproductive toxicity: For the reproductive toxicity endpoint additional requirements for the substructures accompanying the toxophore are given for the following structural alerts:

- Polyalkyl urea
- Monothioglycol or glycol monoalkyl ether, alkoxy- or alkylthio-carboxylic acid or precursors
- Benzidine-based bisazo compound
- Thalidomide-type compound
- Short chain carboxylic acid or precursor
- Pyrroline ester, pyrroline N-oxide ester, pyrrole ester or pyrrole alcohol
- Retinoid or analogue
- Monothioglycol or glycol monoalkyl ether, alkoxy- or alkylthio-carboxylic acid or precursors

For the reproductive toxicity endpoint no domain was given for Triazole antifungal analogue, only a general structural formula, without additional requirements or explanations available. The most detailed domain description is available for 'Short chain carboxylic acid or precursor' and for 'Monothioglycol or glycol monoalkyl ether, alkoxy- or alkylthio-carboxylic acid or precursors'. For other structural alerts only short indications about the possible substituents, required for the alert to fire together with a general structural fragment were given.
Conclusion DEREKfW versus Setubal 2 principle: DEREKfW does contain information about the substituents required for a particular toxophore to be positively identified, but not for all structural alerts. It was concluded, that in general individual structural alerts are not taken into account in isolation, but there are exceptions. For example for the reproductive toxicity alert 'Triazole antifungal analogue'nothing except the general structural formula is given, therefore it is concluded, that this toxophore was taken into account in isolation.
DEREKfW predicts correctly according to its own definitions.

Setubal principle 3: A model should, ideally, have a clear mechanistic basis. In the case of a SAR, there should be a description of the molecular events that underlie the reactivity of the molecule. References should be present (OECD, 2004b). Examples of substances, on which a SAR was based should always be available. Authors of the present report understand 'molecular event' to be the interactions between the molecule and the target receptor/organ.

Comments concern Setubal principle 3:
General comments: A proposal of the mechanistic background is available in DEREKfW in a few cases. Examples of substances that are proven to have a certain toxicological action are presented in some cases.
Specific comments for reproductive toxicity. For the reproductive toxicity endpoint the mechanistic basis is given for two from nine available structural alerts

- short chain carboxylic acid or precursor and
- pyrroline ester, pyrroline N-oxide ester, pyrrole ester or pyrrole alcohol).

Examples are given for three structural alerts:

- Polyalkyl urea,
- Benzidine-based bisazo compound and
- Thalidomide-type compound.

General structural formulas are available for all structural alerts. For all available structural alerts references are available.
Conclusion DEREKfW versus Setubal 3 principle: The description of the mechanistic basis is for several structural alerts not sufficient/adequate enough in DEREKfW and examples of active substances are sometimes missing. On the other hand, references are always present.

Setubal principle 4: A model should be accompanied by a definition of the domain of its applicability. For example the range of physico-chemical properties or chemical classes of chemicals for which it is applicable. In the case of an SAR, information should be given on its applicability to groups of chemicals taking into account if the substructure associated with any inclusion and/or exclusion rules associated with the substructure. In addition the modulatory effects of the substructure's molecular environment should be taken into account. (OECD, 2004b) The authors of the present report understand with the term 'substructure's molecular environment' other activating or deactivating parts in the molecule.

Comments concern Setubal principle 4:
General comments: DEREKfW can assess organic chemicals and some metals. DEREKfW always point out the structural alert, if recognised and if within the chemical domain. Molecular weight and lipophilicity are taken into account for some predications. The presence of other active substituents in the molecule, not directly associated with the structure alert, is sometimes taken into account. For some structural alerts requirements for the whole molecule are given, for some only substituents directly associated with toxophore are taken into account.

The reliability of the predictions is presented in the form of one of the eight levels of likelihood. These levels of likelihood depend on the species for which the prediction was made and in some cases on the molecular weight and/or predicted lipophilicity of the tested molecule. In other words: the level of likelihood is a combination of the presence of the structural fragment, the species in which the defined effect was proved, and the species for which the prediction is required and some physico-chemicals properties. Examples are given in the figures below (Figure 4, 5 and 6). Information on the background of the predictions is given in the form of rules, and the reliability should be judged by careful examination of the related rule.

Specific comments for reproductive toxicity: For reproductive toxicity all positive results were given at 'plausible' likelihood level. Figures 4, 5 and 6 present rules given for each specific endpoint:

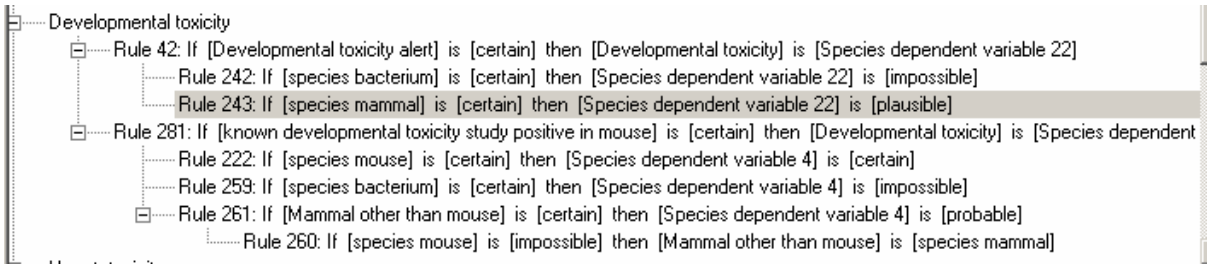

Figure 4: Rules for the developmental toxicity specific endpoint.

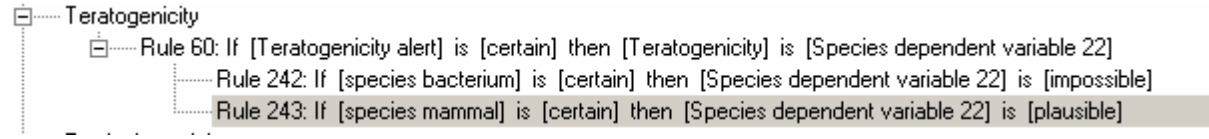

Figure 5: Rules for the teratogenicity specific endpoint.

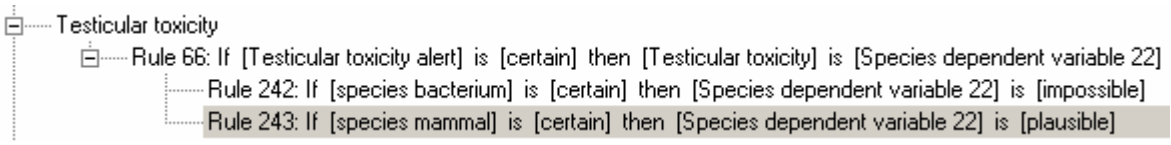

Figure 6: Rules for the testicular toxicity specific endpoint.

For the reproductive toxicity endpoint, as for the other endpoints, activating or deactivating substituents not directly associated with the structural alert were not always taken into account. No substructural environment was given for 'Triazole antifungal analogue'. The most detailed domain description is available for 'Short chain carboxylic acid or precursor' and 'Monothioglycol or glycol monoalkyl ether, alkoxy- or alkylthio-carboxylic acid or precursors'.
Conclusion DEREKfW versus Setubal 4 principle: DEREKfW partly fulfils the Setubal principle 4, as substituents not directly associated with the structural alert were not always taken into account.

Setubal principle 5: A model should be associated with a measure of its goodness-of-fit (internal validation). There should be access to the training and validation data set as well as to the methods used for the development and validation of the model. This training set should include details of chemical names, structural formulas, CAS number (if available) and data for all background information, needed for the reliable interpretation of (Q)SAR (OECD, 2004).
Comments concern Setubal principle 5:
General comments: A training set is not available for DEREKfW. Several positive and negative chemicals are used to establish the SAR, but only some positive examples are available for the user, as some data are confidential. Rules description and references are present in the program. The references contain part of the training set. For example the ECETOC report on glycol ethers is referenced for the alert 'Monothioglycol or glycol monoalkyl ether', giving the training set (ECETOC, 1995). The examples of positive substances are given together with predictions as 'Alert description'. These data are also given in the editor part.

Specific comments for reproductive toxicity: For the reproductive toxicity endpoint, positive examples of chemical on which the SAR is based are given for three structural alerts:

- Polyalkyl urea,
- Benzidine-based bisazo compound and
- Thalidomide-type compound.

No examples are given for the other structural alerts.
Conclusion DEREKfW versus Setubal 5 principle: A the training set is not available for all structural alerts, it was concluded that Setubal principle 4 is only partly fulfilled.

Setubal principle 6: A model should be assessed in terms of its predictive power by using data that were not used in the development of the model (external validation). For validation of models detecting several (eco)toxicological endpoints, the validation should be performed per endpoint. The following parameters should be given:

- the number of test structures;
- the identities of the test structures;
- the approach for selecting the test structures;
- the statistical analysis of the predictive performance of the model (e.g. including sensitivity, specificity, and positive and negative predictions for classification models).

According to the authors of the present paper, the external validation of a SAR model should not be performed for a model in general. More specific goals should be defined, for example the validation of a SAR model should be conducted for a well defined endpoint containing well defined structural alerts, e.g. the following validation set may be proposed:
- positive substances, containing tested SAR;
- negative substances, containing tested SAR;
- negative substances, not containing tested SAR.

Comments concern Setubal principle 6:
General comments: As there is quite some debate on the principles of external validation, we prefer the word performance instead of validation. The performance of DEREKfW for reproductive toxicity on industrial chemicals was one of the goals of this project. This performance was carried out for the reproductive toxicity end point using 108 substances. All identities of tested structures are presented in Appendix 1. The approach for selecting substances is given in chapter 2, 'Methods'.

3.2 Performance of DEREKfW and the TSCA Chemical Category List

3.2.1 The test set

From all the Annex 1 substances (EC, 2004b), 108 substances labelled with R60, R61, R62, R63 and R64 were initially chosen for this test (listed in Appendix 1, excluding all non-organic chemicals). No chemicals were chosen that are not classified for reproductive effect. As DEREKfW and the TSCA Chemical Category List do not contain the structural alert for effects during lactation, substances labelled with R64 only (three substances) were excluded from this performance. For the DEREKfW performance substances classified for reproductive toxicity were considered to be correctly recognised, if they were not only recognised as reprotoxic chemicals, but also placed in the correct category (impaired fertility

or developmental toxicity; see comparison of classification in DEREKfW and Appendix 1, Table 3) For the TSCA Chemical Category performance the same approach was used (see comparison of classification in TSCA Chemical Category List and Appendix 1, Table 4), except for the term 'reproductive toxicity'. This term was understood as both impairing fertility and harmful to unborn child, if no further specification in TSCA Chemical Category List was given.

From the remaining group of 105 substances, DEREKfW (Table 6) correctly predicted 13 substances containing alerts for teratogenicity or developmental or testicular toxicity. Three of the detected substances were used as positive examples (benzidine-based bisazo compounds) and therefore were left out from the evaluation of the results, leaving 102 substances for the evaluation. For the performance of the TSCA Chemical Category List 105 substances were used. The references used by DEREKfW for deriving the structural alerts can contain chemicals used for the training set. These references have not been searched systematically and chemicals given in these references were not left out. For example, DEREKfW refers to the ECETOC report on glycolethers and this report contains several chemicals of the Annex 1 (ECETOC, 1995 and EC, 2001a, respectively). The training set of TSCA Chemical Category List is unknown.

Some of the substances were classified with more than one risk sentence, for example ethylene glycol monomethyl ether (CAS no. 109-86-4) is classified with R60 and R61 for both fertility and developmental toxicity. Therefore the total number of chemicals in the Table 5 exceeds 102 for the DEREKfW performance and 105 for the TSCA Chemical Category List performance (Table 7).

To summarise, the performance set of the Annex I substances differed between the tested models, as the models have different predictive capacity (both fertility and teratogenicity or teratogenicity only), variable specificity of predictions (for the TSCA Chemical Category List further clarification for the term 'reproductive toxicity' was not available in some cases) and different training sets.

It needs to be stressed that this test set only has positive industrial chemicals. The sensitivity can only be expressed for the whole test set. Sensitivity also needs to be assessed for each endpoint. As we tested two-four endpoints:
- impaired fertility (R60 and R62) and
- harm to the unborn child (R61 and R63),

the number of predictions exceeds the number of chemicals (Table 6).

3.2.2 Performance of DEREKfW

The percentage of predictions positively classified for 'impaired fertility' was 10% (6/57) (Table 6.). For 'harm to the unborn child this value was 19% (13/69) (Table 6). Most of the positively predicted substances were ethylene glycol ethers. Furthermore, one short chain carboxylic acid was recognised. For substances labelled with the R60 and R61 (i.e. substances known to impair fertility or to cause developmental toxicity in humans**,** and substances which should be regarded as if they impair fertility or cause developmental toxicity in humans; category 1 and 2), the frequency of detection was higher than for substances classified as R62 and R63 (i.e. substances**,** which cause concern for human fertility or cause concern for humans owing to possible developmental effects; cat.3).

In conclusion, substances with a more severe classification were recognised more often than substances with a less severe classification. In addition, the predictive value of DEREKfW was marginally better when assessing the effects on development (19% correct compared to the effects on fertility 10%). In Table 6 an overview of the results is presented.

DEREKfW could not be tested for several of its structural alerts, because no members of these groups were present in the Annex I or the working database for 29th ATP:
- Thalidomide-type compounds (Teratogenicity),
- Pyrroline ester, pyrroline N-oxide ester, pyrrole ester or pyrrole alcohol (Teratogenicity)
- Triazole antifungal analogue (Teratogenicity)
- Retinoid or analogue (Teratogenicity)
- Polyalkyl urea (Developmental toxicity)

The detailed list of substances recognised as toxic to reproduction by DEREKfW, including the grounds for prediction (structural alert) is presented in the table below. All the positive DEREKfW results were predicted at the likelihood level 'plausible' in this study.

Table 6: DEREKfW reproductive toxicity correlation (102 chemicals).

Classification	Number of substances classified in the database [1]	Number of substances correctly predicted by DEREKfW[2]	Percentage of correctly predicted positive substances (Sensitivity)	Percentage of false negative predictions
Fertility (DEREKfW SAR: testicular toxicity)				
R60 (May impair fertility)	19	5	26%	74%
R62(Possible risk of impaired fertility)	38	1	3%	97%
R60 or R62	**57**	**6**	**10%**	**90%**
Developmental toxicity (DEREKfW SAR: teratogenicity or developmental toxicity)				
R61 (May cause harm to the unborn child)	42	9	21%	79%
R63 (Possible risk of harm to the unborn child.)	27	4	4%	96%
R61 or R63	**69**	**13**	**19%**	**81%**

1 As some of the substances were classified with more than one risk sentence, the total number of chemicals exceeds 102.

2 Substances classified for reproductive toxicity was considered to be correctly recognised, if recognised not only as reprotoxic chemicals, but also placed in the correct category: impaired fertility versus developmental toxicity (see comparison of classification in DEREKfW and Annex 1, Table 3).

Table 7 DEREKfW predictions concerning reprotoxic substances (Annex 1 substances, classified with R60, R61, R62 and/or R63).

No.	Substance chemical name, CAS no. and classification	Results from DEREKfW		Structural alert defined in DEREKfW (chemical class)
		Testicular toxicity	Developmental toxicity	
1.	2-methoxyethanol, ethylene glycol monomethyl ether CAS 109-86-4 Fertility: R60 Developmental: R61	Testicular toxicity: plausible	Developmental toxicity: plausible	Testicular and developmental toxicity: Monothioglycol or glycol monoalkyl ether, alkoxy- or alkylthio-carboxylic acid or precursors
2.	2-ethoxyethanol, ethylene glycol monoethyl ether CAS 110-80-5 Fertility: R60 Developmental: R61	Testicular toxicity: plausible	Developmental toxicity: plausible	Testicular and developmental toxicity: Monothioglycol or glycol monoalkyl ether, alkoxy- or alkylthio-carboxylic acid or precursors
3.	2-methoxyethyl acetate, methylglycol acetate CAS 110-49-6 Fertility: R60 Developmental: R61	Testicular toxicity: plausible	Developmental toxicity: plausible	Testicular and developmental toxicity: Monothioglycol or glycol monoalkyl ether, alkoxy- or alkylthio-carboxylic acid or precursors
4.	2-ethoxyethyl acetate, ethylglycol acetate CAS 111-15-9 Fertility: R60 Developmental: R61	Testicular toxicity: plausible	Developmental toxicity: plausible	Testicular and developmental toxicity: Monothioglycol or glycol monoalkyl ether, alkoxy- or alkylthio-carboxylic acid or precursors
5.	Bis(2-methoxyethyl) phthalate CAS 117-82-8 Fertility: R62 Developmental: R61	Testicular toxicity: plausible	Developmental toxicity: plausible	Testicular and developmental toxicity: Monothioglycol or glycol monoalkyl ether, alkoxy- or alkylthio-carboxylic acid or precursors
6.	6-(2-chloroethyl)-6-(2-methoxyethoxy)-2,5,7,10-tetraoxa-6-silaundecane, etacelasil CAS 37894-46-5 Fertility: - Developmental: R61	Testicular toxicity: plausible	Developmental toxicity: plausible	Testicular and developmental toxicity: Monothioglycol or glycol monoalkyl ether, alkoxy- or alkylthio-carboxylic acid or precursors
7.	Methoxyacetic acid CAS 625-45-6 Fertility: R60 Developmental: R61	Testicular toxicity: plausible	Developmental toxicity: plausible	Testicular and developmental toxicity: Monothioglycol or glycol monoalkyl ether, alkoxy- or alkylthio-carboxylic acid or precursors
8.	2-methoxypropanol CAS 1589-47-5 Fertility: - Developmental: R61		Developmental toxicity: plausible	Developmental toxicity: Monothioglycol or glycol monoalkyl ether, alkoxy- or alkylthio-carboxylic acid or precursors
9.	2-methoxypropyl acetate CAS 70657-70-4 Fertility: - Developmental: R61 Lactation: -		Developmental toxicity: plausible	Developmental toxicity: Monothioglycol or glycol monoalkyl ether, alkoxy- or alkylthio-carboxylic acid or precursors
10.	2-ethylhexanoic acid CAS 149-57-5 Fertility: - Developmental: R63		Teratogenicity: plausible	Teratogenicity: short chain carboxylic acid or precursor

3.2.3 Performance of TSCA Chemical Categories

The percentage of the predictions positively classified for 'impaired fertility' 23% (13/57). For 'harm to the unborn child this value was 18% (13/72) (Table 8). Most of the correctly predicted substances were ethylene glycol ethers and epoxides. Similarly to DEREKfW, for substances labelled with R60 and R61 (substances known to impair fertility or to cause developmental toxicity in humans**,** and substances which should be regarded as if they impair fertility or cause developmental toxicity in humans; category 1 and 2), the frequency of detection was higher than for substances with the R62 and R63 classification (substances**,** which cause concern for human fertility or cause concern for humans owing to possible developmental effects; category 3).

In conclusion, substances with a more severe classification were recognised more often than the substance with a less severe classification. In addition, the predictive value of TSCA Chemical Categories list was a slightly greater when considering the effects on fertility with 23% of predictions being correct compared 18% for the effects on development. In Table 8 an overview of the results is presented.

Table 8: TSCA Chemical Categories reproductive toxicity correlation (105 chemicals).

Classification	Number of classified substances the database [1]	Number of substances correctly predicted by TSCA[2]	Percentage of correctly predicted positive substances (Sensitivity)	Percentage of false negative predictions
Fertility **(TSCA SAR (Chemical Category):** Toxic to male reproductive system, Sterility in males and females, Testicular atrophy in males, Reproductive toxicity[3]**)**				
R60 (May impair fertility)	19	8	42%	58%
R62(Possible risk of impaired fertility)	38	5	13%	87%
R60 or R62	**57**	**13**	**23%**	**77%**
Developmental toxicity **(TSCA SAR (Chemical Category):** Fetotoxicity, Reproductive toxicity[3], Developmental toxicity**)**				
R61 (May cause harm to the unborn child)	42	10	23%	77%
R63 (Possible risk of harm to the unborn child.)	30	3	10%	90%
R61 or R63	**72**	**13**	**18%**	**82%**

1 As some of the substances were classified with more than one risk sentence, the total number of chemicals exceeds 105.

2 Substance classified for reproductive toxicity was considered to be correctly recognised, if recognised not only as reprotoxic chemicals, but also placed in the correct category: impaired fertility versus developmental toxicity (see comparison of classification in TSCA Chemical Category List and Annex 1, Table 4).

3 If no further specification in TSCA Chemical Category List was given, the term 'reproductive toxicity' was understood as both impairing fertility and harmful to unborn child.

The detailed list of substances recognised as toxic to reproductive using TSCA Chemical Categories List, including grounds for prediction (structural alert/Chemical Category definition) is presented in the table below.

Table 9 Substances recognised as reprotoxic using TSCA Chemical Category List (Annex 1 substances, classified with R60, R61, R62 and/or R63).

No.	Substance chemical name, CAS no. and classification	Classified according to the TSCA List as a member of the following Chemical Category
1.	Acrylamide, prop-2-enamide; CAS No.: 79-06-1 Fertility: R62	Acrylamides: reproductive and developmental toxicants
2.	4,4'-oxydianiline [1] and its salts, p-aminophenyl ether; CAS No.: 101-80-4 Fertility: R62	Di-anilines: potential reproductive toxicants by analogy to 4,4'-methylenedianiline
3.	Allyl 2,3-epoxypropyl ether, allyl glycidyl ether, prop-2-en-1-yl 2,3-epoxypropyl ether; CAS No.: 106-92-3 Fertility: R62	Epoxides: reproductive effects
4.	2,3-epoxypropan-1-ol, glycidol; CAS No.: 556-52-5 Fertility: R60	
5.	R-2,3-epoxy-1-propanol; CAS No.: 57044-25-4 Fertility: R60	
6.	(2RS,3RS)-3-(2-chlorophenyl)-2-(4-fluorophenyl)-[(1H-1,2,4-triazol-1-yl)methyl]oxirane, epoxiconazol; CAS No.: 133855-98-8 Fertility: R62 Developmental: R63	
7.	(2RS,3RS)-3-(2-chlorophenyl)-2-(4-fluorophenyl)-[(1H-1,2,4-triazol-1-yl)methyl]oxirane; CAS No.: 106325-08-0 Developmental: R61	
8.	2-methoxyethanol, ethylene glycol monomethyl ether; CAS No.: 109-86-4 Fertility: R60 Developmental: R61	Ethylene Glycol Ethers: developmental and reproductive toxicants.
9.	2-ethoxyethanol, ethylene glycol monoethyl ether; CAS No.: 110-80-5 Fertility: R60 Developmental: R61	
10.	2-(2-methoxyethoxy)ethanol, diethylene glycol monomethyl ether; CAS No.: 111-77-3 Developmental: R63	
11.	Bis(2-methoxyethyl) ether; CAS No.: 111-96-6 Fertility: R60 Developmental: R61	
12.	1,2-Bis(2-methoxyethoxy)ethane, TEGDME, Triethylene glycol dimethyl ether, Triglyme; CAS No.: 112-49-2 Fertility: R62 Developmental: R61	
13.	1,2-dimethoxyethane, EGDME, ethylene glycol dimethyl ether; CAS No.: 110-71-4 Fertility: R60 Developmental: R61	

No.	Substance chemical name, CAS no. and classification	Classified according to the TSCA List as a member of the following Chemical Category
14.	2-methoxyethyl acetate, methylglycol acetate; CAS No.: 110-49-6 Fertility: R60 Developmental: R61	
15.	2-ethoxyethyl acetate, ethylglycol acetate; CAS No.: 111-15-9 Fertility: R60 Developmental: R61	
16.	Bis(2-methoxyethyl) phthalate; CAS No.: 117-82-8 Fertility: R62 Developmental: R61	
17.	6-(2-chloroethyl)-6-(2-methoxyethoxy)-2,5,7,10-tetraoxa-6-silaundecane, etacelasil; CAS No.: 37894-46-5 Developmental: R61	
18.	Malachite green hydrochloride (A) [1]; malachite green oxalate (B) [2]; CAS No.: 569-64-2 [1]; 18015-76-4 [2] Developmental: R63	Triarylmethane Pigments/Dyes with Non-solubilizing Groups: developmental and reproductive toxicity

The TSCA Chemical Categories List could not be tested for several of its structural alerts, because no members of these groups were present in Annex I or the working database for 29th ATP (EC, 2004b):

- Anhydrides of Carboxylic Acid
- Benzotriazole-hindered phenols
- Boron Compounds: reproductive toxicity (i.e., sterility in males and females, and testicular atrophy in males).
- Hindered Amines: toxic to male reproductive system
- Nickel Compounds: fetotoxicity
- Vinyl Esters: Reproductive toxicity

The identification accuracy of a Chemical Categories member depends greatly on the background and the experience of the person performing the investigation. Some classes are described in a detailed way and a certain chemical knowledge is required for the correct recognition of the functional groups and substituents. For example for ethylene glycol ethers substituent R' may be 'any group that can be chemically or metabolically removed to yield a glycol ether'. Therefore, there is a possibility that other investigators would obtain different results from those presented here, despite using the same Annex 1 list.

3.2.4 Comparison of structural alerts from the different (Q)SAR sources.

Ethylene glycol ethers. DEREKfW recognised 9 substances as ethylene glycol ethers, while 10 substances were identified as members of this group using TSCA Chemical Categories definition. Four substances were recognised as glycol ethers by both DEREKfW and TSCA Chemical Category List. The percentage of correct predictions of DEREKfW for the ethylene glycol ethers depends upon the definition of the structural alert/toxophore and domain. The DEREKfW definition is for mono- or diethers. Therefore, DEREKfW does not recognise the glycol ethers with three glycol ether groups (on one chain, and not branched). According to the TSCA Chemical Category List definition, the glycol ethers with three glycol ether groups

are identified as reprotoxic. DEREKfW allows the inclusion of side chains on the ethylene ether moiety (-OCH2-CH2-), whereas the TSCA Chemical Category List does not.

The DEREKfW structural alert for 'Monothioglycol or glycol monoalkyl ether, alkoxy- or alkylthio-carboxylic acid or precursors' is based on one reference for both developmental toxicity and testicular toxicity (ECETOC Technical Report). No examples are given.

TSCA Chemical Categories List contains category 'Ethylene glycol ether'. The definition includes several detailed requirements, together with requests for specific substituents.

Carboxylic acids. DEREKfW recognised one carboxylic acid. Another substance also containing the carboxylic group (methoxyacetic acid) was only recognised because a ethylene glycol ether group was also present. DEREKfW contains an alert for carboxylic acids with very specific requirements regarding branching and side-chains. The DEREKfW structural alert, for 'Short chain carboxylic acid or precursor' for teratogenicity, is based on 6 references. No examples are given.

The TSCA List contains only the Chemical Category 'Carboxylic acid anhydride', and not carboxylic acid itself.

Epoxides. Following the TSCA Chemical Categories definitions, 5 epoxides were recognised. This definition is further not specified, every molecule containing an epoxide substituent is considered to be a member of this category. DEREKfW does not contain structural alerts for reproductive toxicity of epoxides (epoxides are recognised by DEREKfW as skin and eye irritants).

Acrylamides. Following the TSCA Chemical Categories definitions, one acrylamide was recognised. DEREKfW does not contain structural alerts for reproductive toxicity of acrylamides (a structural alert for acrylamide for neurotoxicity is present in DEREKfW).

Di-anilines. Following the TSCA Chemical Categories definitions, one di-aniline was recognised. This definition includes several detailed requirements, together with requests for specific substituents. DEREKfW does not contain structural alerts for di-anilines.

Triarylmethane Pigments/Dyes with Non-solubilizing Groups. Following the TSCA Chemical Categories definitions, one triarylmethane pigment was recognised. This definition includes a general structural formula of this group of substances. DEREKfW does not contain structural alerts for Triarylmethane Pigments/Dyes with Non-solubilizing Groups.

Table 10: Comparison of DEREKfW and TSCA Chemical Category predictions for reproductive toxicity.

Substance	Recognised by DEREKfW	Recognised using TSCA
2-methoxyethanol, ethylene glycol monomethyl ether, CAS 109-86-4	Monothioglycol or glycol monoalkyl ether, alkoxy- or alkylthio-carboxylic acid or precursors	Ethylene Glycol Ethers
2-ethoxyethanol, ethylene glycol monoethyl ether, CAS 110-80-5	Monothioglycol or glycol monoalkyl ether, alkoxy- or alkylthio-carboxylic acid or precursors	Ethylene Glycol Ethers
2-methoxyethyl acetate, methylglycol acetate, CAS 110-49-6	Monothioglycol or glycol monoalkyl ether, alkoxy- or alkylthio-carboxylic acid or precursors	Ethylene Glycol Ethers

Substance	Recognised by DEREKfW	Recognised using TSCA
2-ethoxyethyl acetate, ethylglycol acetate, CAS 111-15-9	Monothioglycol or glycol monoalkyl ether, alkoxy- or alkylthio-carboxylic acid or precursors	Ethylene Glycol Ethers
Bis(2-methoxyethyl) phthalate, CAS 117-82-8	Monothioglycol or glycol monoalkyl ether, alkoxy- or alkylthio-carboxylic acid or precursors	Ethylene Glycol Ethers
6-(2-chloroethyl)-6-(2-methoxyethoxy)-2,5,7,10-tetraoxa-6-silaundecane, etacelasil, CAS 37894-46-5	Monothioglycol or glycol monoalkyl ether, alkoxy- or alkylthio-carboxylic acid or precursors	Ethylene Glycol Ethers
Methoxyacetic acid, CAS 625-45-6	Monothioglycol or glycol monoalkyl ether, alkoxy- or alkylthio-carboxylic acid or precursors	-
2-methoxypropanol, CAS 1589-47-5	Monothioglycol or glycol monoalkyl ether, alkoxy- or alkylthio-carboxylic acid or precursors	-
2-methoxypropyl acetate, CAS 70657-70-4	Monothioglycol or glycol monoalkyl ether, alkoxy- or alkylthio-carboxylic acid or precursors	-
2-(2-methoxyethoxy)ethanol, diethylene glycol monomethyl ether; CAS No.: 111-77-3	-	Ethylene Glycol Ethers
Bis(2-methoxyethyl) ether; CAS No.: 111-96-6	-	Ethylene Glycol Ethers
1,2-Bis(2-methoxyethoxy)ethane, TEGDME, Triethylene glycol dimethyl ether, Triglyme; CAS No.: 112-49-2	-	Ethylene Glycol Ethers
1,2-dimethoxyethane, EGDME, ethylene, glycol dimethyl ether; CAS No.: 110-71-4	-	Ethylene Glycol Ethers
2-ethylhexanoic acid, CAS 149-57-5	Short chain carboxylic acid or precursor	-
Allyl 2,3-epoxypropyl ether, allyl glycidyl ether, prop-2-en-1-yl 2,3-epoxypropyl ether; CAS No.: 106-92-3	-	Epoxides
2,3-epoxypropan-1-ol, glycidol; CAS No.: 556-52-5	-	Epoxides
R-2,3-epoxy-1-propanol; CAS No.: 57044-25-4	-	Epoxides
(2RS,3RS)-3-(2-chlorophenyl)-2-(4-fluorophenyl)-[(1H-1,2,4-triazol-1-yl)methyl]oxirane, epoxiconazol; CAS No.: 133855-98-8	-	Epoxides
(2RS,3RS)-3-(2-chlorophenyl)-2-(4-fluorophenyl)-[(1H-1,2,4-triazol-1-yl)methyl]oxirane; CAS No.: 106325-08-0	-	Epoxides
Acrylamide, prop-2-enamide;CAS No.: 79-06-1	-	Acrylamides
4,4'-oxydianiline [1] and its salts, p-aminophenyl ether; CAS No.: 101-80-4	-	Dianilines
Malachite green hydrochloride (A) [1]; malachite green oxalate (B) [2]; CAS No.: 569-64-2 [1]; 18015-76-4 [2]	-	Triarylmethane, Pigments /Dyes with Non-solubilizing Groups

4. Discussion and recommendations

The foregoing chapters will be discussed focussing on the following two issues:
1) Application of the OECD (Q)SAR principles to DEREKfW 6.0 (OECD, 2004b), in a general way and for the reproductive toxicity endpoint, specifically.
2) Comparing reproductive SARs in DEREKfW and the TSCA Chemical Category List with experimental data.

For testing of (Q)SAR computer models (4.3) chemicals, classified in EU as reprotoxic, based mostly on animal tests, described in Annex 1 (EC, 2001a; EC 2004b) were used. The advantages and limitation of the chosen test set will be discussed. Finally, recommendations for improvement of DEREKfW and the TSCA Chemical Category List will be given and further work will be addressed (4.4). In the last section the final conclusions will be drawn (4.5)

4.1 OECD (Q)SAR principles

For principle 1 (defined endpoint), endpoints are indeed established in the references, but the detailed definition is not given in the manual or programme For example, for testicular toxicity the endpoint is clear and could directly be translated to reproductive toxicity according to EU classification. For developmental toxicity the references had to be checked for the defined endpoint e.g. growth retardation or malformation. For our exercise we could just translate developmental toxicity from DEREKfW to EU developmental toxicity classification (see Table 3 and Table 4). It is concluded, that the principle 1 is partly fulfilled.

The second principle (clear descriptors and hierarchy) was judged as partly fulfilled. DEREKfW contains requirements specifying the substituents of the structural formula, which should accompany the structural alert. The general impression for the whole program is that this is true for some of the structural alerts, but not for all. For the reproductive toxicity endpoint 8 from 9 available structural alerts were sufficiently described. For the reproductive toxicity endpoint un unclear domain was given for ‘Triazole antifungal analogue’, it seems like a general structural formula, without additional requirements for substituents is available. DEREKfW predictions are consequent within the domains given.

Setubal principle 3 (clear mechanistic basis if possible) is partly fulfilled, as structural alerts and corresponding references are available. The description of the mechanistic basis is given for some structural alerts, but at times insufficiently. Examples of active substances are sometimes missing. For 2 from 9 structural alerts for reproductive toxicity, mechanistic basis is given in the Editor Part of DEREKfW. A clear indication of the structural alert makes the prediction understandable, and the presence of references allows the verification of the prediction.

The fourth principle (defined domain of applicability) was fulfilled to a certain extent. For some structural alerts requirements concerning the whole molecule are given (for example ‘short chain carboxylic acid or precursors’). For other SARs more general description is available. The influence of other active substituents, not directly associated with the structure alert is sometimes lacking. The deficiency of this association may have consequences for the more complex molecules. Some active groups may have antagonistic action, resulting in no effect at all. Other active groups may enhance the effect.

Principle five (internal validation, training set available) was considered to have limited coverage as only part of the training set is available in the references; no more than some key examples of positive chemicals relevant to a particular alert. Presence of an example of a positive substance allows the user to compare the chemical structure under evaluation with a given active substance. The reference is indeed always present. However, some of the background literature is rather old and not easily obtainable.

It was concluded, that DEREKfW fulfils partly the OECD (Q)SAR principles one to five for the reproductive toxicity endpoint (Table 11).

Table 11: DEREKfW versus OECD (Q)SAR principles.

Setubal principle	Fulfilled by DEREKfW
1. Defined endpoint	Partly
2. Clear descriptors and hierarchy	Partly
3. Clear mechanistic basis	Partly
4. Domain	Partly
5. Internal validation	Partly

Principle 6 (external validation). Pearl et al. (2001) compared in-vitro teratogenicity data with DEREKfW predictions. They present the performance study of DEREKfW, together with TOPKAT and MULTICASE. Identities of the tested structures are not given and the approach for selecting structures was unknown. The following results for DEREKfW were obtained. They used both non-teratogenic and teratogenic chemicals.

Table 12: Predictability of DEREKfW for teratogenicity (Pearl et al., 2001).

Endpoint and test set	Accuracy*	False Positive	False negative	Intermediate
Teratogenicity, 34 positive rodent teratogens, and 71 negative teratogens.	72%	0%	28%	-

***** Accuracy is the percentage of correct outcomes of a method.

Pearl et al. (2001) showed that DEREKfW identified only 10 from 34 positive *in vivo* teratogens (Table 12). All identified substances were retinoic acids. The conclusions of the authors were, that at present DEREKfW is not able to give reliable predictions for in-vivo teratogenicity. In our performance study DEREKfW also showed a limited sensitivity (10 and 19% for 'impaired fertility' and 'harm to the unborn child', respectively). DEREKfW detected mainly 'ethyleneglycolethers'.

Although the performance of DEREKfW has been tested for chemicals unlikely being used for developing the model, the results show that DEREKfW can highlight some reproductive chemicals but shows currently a limited coverage for the two reproductive toxic endpoints tested here. In other words the applicability domain of DEREKfW is limited.

Discussion concerning OECD (Q)SAR principles:

Principle one, the defined endpoint in DEREKfW, can be interpreted as being partly fulfilled as the endpoints are defined and literature references are given for further details. DEREKfW uses different literature data for deriving the alerts. There is not a specific test method e.g. according to OECD guidelines underlying the predictions made for the different endpoints.

Principle 2 and 4 could not be easily distinguished for structural alert description. In both cases it might be understood, that also other substituents, next to the structural alert itself,

should be taken into account. A distinction may be made between substituents accompanying the structural alert directly and other active substituents present in the molecule that may influence the toxicity.

4.2 Comparing positive SARs with positive reproductive experimental data

Both DEREKfW and the TSCA Chemical Category List recognised only a low percentage of the substances classified for reproductive toxicity (i.e. showed low sensitivity). The percentage of the tested substances correctly classified for 'impaired fertility' was 10% (6/57) (Table 6.). For 'harm to the unborn child this value was 19% (13/69) (Table 6). To express these results in the scope of REACH, DEREKfW missed 90% and 81%, respectively. Applying the chemical categories of the TSCA-List, the following percentages were found. For 'impaired fertility' this was 23% (13/57). For 'harm to the unborn child this value was 18% (13/72) (Table 6). When we want to use the TSCA Chemical Category List in the scope of REACH, 77% and 82%, of these reproductive toxic chemical were missed, though the chemicals identified may limit further testing using the information in the models. At the other hand every identified chemical identified may need no or limited further testing using the information available in the models. The chemicals in the (partially unknown) training set were not excluded from this exercise and therefore the predictions may even be overestimated. For DEREKfW only the positive examples given were left out of the test set. Despite the failure of the models to predict most the EU classified chemicals we did some remarkable observations.

The first striking feature was that DEREKfW and the TSCA Chemical Category List only had one structural alert in common, i.e. 'monothioglycol monoalkyl ether, alkoxy- or alkylthio-carboxylic acid or precursors' and 'ethylene glycol ether', respectively. Moreover the models showed only a partial overlap in their predictions for 'ethylene glycol ethers. Six chemicals with these similar alerts were selected by both models (Table 10). The applicability domains of each model are apparently different for these similar alerts. In addition, the authors of the present report 'recognised' more 'ethylene glycol ethers' in the test set, without having a specific applicability domain in mind. Using this test set with only positive reproductive chemicals it is not possible to evaluate the applicability domains of the models. To establish the domain, positive and negative chemicals, containing a certain alert, need to be available. DEREKfW recognised all the 'monothioglycol monoalkyl ether, alkoxy- or alkylthio-carboxylic acid or precursors' within its own applicability domain. Evaluating the performance using the applicability domain of DEREKfW, we can only find false positives if we test chemicals that contain the alert and are negative in the experimental test. These chemicals were not included in the test set.

The second feature was that besides the ethyleneglycolethers, DEREKfW contains seven, and the TSCA Chemical Category List 10 additional reproductive toxicity alerts without any overlap (see table 13). Therefore a reason for the low sensitivity is probably the limited number of alerts established for the reproductive endpoint in general and/or included in the models.

A third observation was that the highest percentage of correctly predicted substances causes the clearest and most severe toxic effects. These substances are labelled with R60 (May impair fertility). DEREKfW and the TSCA Chemical Category List correctly identified 26% and 42%, respectively, of the chemicals classified with R60 (Table 6 and 8, respectively).

DEREKfW predicted only 3% of the substances labelled with R62 (Possible risk of impaired fertility) (Table 6).The difference in the accuracy of predictions between the more severe and less severe labelling is likely to be due to the more obvious or better proven effects for more severely labelled substances resulting in better defined and known structural alerts.

The fourth remark that can be made is that not for all structural alerts in DEREKfW and categories in the TSCA Chemical Category List and substances from Annex 1 was available. This means, that several SARs in the models were not tested. In addition, for most groups where an alert was present in DEREKfW or the TSCA Chemical Category List, no firm conclusions can be made, because the number of substances available for testing the individual structural alerts was very limited.

Summarising: Only for the ethylene glycol ethers, the structural alert present in both models was there a sufficient number of examples in Annex 1 allowing performance of a more detailed evaluation, which is discussed in the first paragraph of this subchapter.

Table 13 Comparison of the structural alerts for the reproductive toxicity endpoints from DEREKfW and the TSCA Chemical Category List.

Structural alerts present in DEREKfW	Structural alerts present in TSCA-List
Polyalkyl urea #	
Benzidine-based bisazo compound **(3)**	
Thalidomide-type compound #	
Short chain carboxylic acid or precursor **(1)**	
Pyrroline ester, pyrroline N-oxide ester, pyrrole ester or pyrrole alcohol #	
Triazole antifungal analogue #	
Retinoid or analogue #	
Monothioglycol or glycol monoalkyl ether, alkoxy- or alkylthio-carboxylic acid or precursors **(9)**	Ethylene Glycol Ethers **(10)**
	Acrylamides **(1)**
	Anhydrides, Carboxylic Acid #
	Dianilines **(1)**
	Benzotriazole-hindered phenols #
	Boron Compounds #
	Epoxides **(5)**
	Hindered Amines #
	Nickel Compounds #
	Triarylmethane Pigments/Dyes with Non-solubilizing Groups **(1)**
	Vinyl Esters #

() = The number of substances predicted positive
= No substances present for this alert

To summarise the low predictive performance of DEREKfW and the TSCA Chemical Category List obtained by our investigation can be explained as follows:

- A limited number of structural alerts in general for reproductive toxicity and incorporated in DEREKfW and the TSCA Chemical Category List for reproductive toxicity
- A difference in domain definition: the TSCA Chemical Category List identified different glycol ethers as compared to DEREKfW.
- No EU classified chemicals for reproductive toxicity were detected for 5 and 4 structural alerts for DEREKfW and TSCA Chemical Category list, respectively (metals were not included in our evaluation). Therefore the predictive performance of DEREKfW and TSCA Chemical Category List is partially tested.

4.3 Additional comments concerning DEREKfW and TSCA Chemical Category List

We conclude that DEREKfW is a comprehensible model with a transparent structure. Grounds for the prediction are present and references are given; therefore the reliability of every prediction can be verified. Some experience with reading the rules is needed. For deriving the publicly available training set the contents of these references have to be evaluated. In addition, by using the editor part of the program, additional information on the content of the model can be obtained. The screen lay-out is clear and all available information is obtainable. DEREKfW assesses the structural alert and the DEREKfW domain for you, which is on one hand more time efficient and more consistent, than assessing the TSCA Chemicals Category List manually. The TSCA Chemical Category List is a useful compilation of accepted structural alerts derived by a group of experts for various endpoints.

4.4 Validity of the test set

The list of reproductive substances from Annex I was chosen as a test set, because this list contains industrial substances that have been evaluated according to one set of criteria by the same experts. The data are not confidential and information on most substances is available on the ECB website (ECB, 2004b) for example CAS number, chemical name and complete classification.
Limitations with using this list to validate (Q)SAR prediction include the following:

- Only positive industrial substances were tested;
- Not all structural alerts present in DEREKfW were tested, as for some there were no examples from Annex 1 available;
- Only limited amount of positive examples were available for some structural alerts.

The reproductive substances list of Annex 1 is the only available list with reliable reproductive data, easily accessible for everybody and relevant for REACH. Therefore, even taking into account the limitations presented above this performance test provides insight in a) the ‘structural alerts present in the models, b) the presence of these alerts in the EU classified reproductive toxic industrial chemicals and allows to learn how to deal with the OECD (Q)SAR principles when evaluating the models.

4.5 Recommendations

To improve DEREKfW and the TSCA Chemical Category List the authors of the present report recommend the following:

- The important gaps in DEREKfW and the TSCA Chemical Category List were identified as the limited number of structural alerts and incomplete domain definitions. It may be concluded, that both models may be improved by adding known structural alerts, in this case for the reproductive toxicity endpoint. Firstly each model may be supplemented by adding the structural alerts from the other model. Both models have the only one mutual structural alert (ethylene glycol ethers), but even for this alert there are differences in the domain description.
- Some of the references present in DEREKfW and the TSCA Chemical Category List are rather old and therefore sometimes less reliable or not easily obtainable. Using recent

references/data for the describing of the domains would improve reliability of the predictions and make the models more user friendly.

4.5.1 Recommendations for further work

At present, there is no complete collection of SARs for the reproductive endpoint. Reproductive toxicology is very complex and has several different and usually unknown mechanisms. Knowledge about this process is limited and SARs are difficult to define. An attempt to collect and describe the published structural alerts for the reproductive toxicity can be found in Hulzebos et al. (1999, 2001). It would be worthwhile to explore this area further. The predictions of TOPKAT and Multicase for the same performance set will be reported next year in co-operation with the Danish EPA.

4.6 Final conclusions

DEREKfW did not recognize 90 % of the substances classified for ‘impaired fertility’ and 79% of the substances classified for ‘harm to the unborn child’. The TSCA Chemical Category List missed 77% and 82% of the cases, respectively. These values might still be too positive as the training set was partially unknown. Most chemicals were not detected by either method, due to the limited number of structural alerts available and the complex mechanisms of reproductive toxicity. Besides one mutual ‘alert/category’ DEREKfW and TSCA contain 7 ‘alerts’ and 10 categories, respectively. As the alerts in DEREKfW (comprehensible and transparent tool) and categories in the TSCA Chemical Category List are highlighted in this research, they both can be used as additional expert judgment when assessing chemicals for reproductive toxicity. However, we conclude that these models cannot be the only method for screening chemicals for reproductive toxicity in the framework of REACH. Other models or testing strategies have to be used to assess reproductive toxicity of chemicals.

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Appendix 1 Reproductive classified chemicals

Chemicals classified for reproductive toxicity according to Annex I and working database of the 29th ATP (according to Directive 67/548/EC) used for comparing the predictions of DEREK and TSCA with this classification.

Chemical name	CAS number	Classification
Carbon disulphide	75-15-0	R: **62-63**
6-(2-chloroethyl)-6-(2-methoxyethoxy)-2,5,7,10-tetraoxa-6-silaundecane, etacelasil	37894-46-5	R: **61**
Bis(4-fluorophenyl)(methyl)(1H-1,2,4-triazol-1-ylmethyl)silane, flusilazole (ISO)	85509-19-9	R: **61**
Octamethylcyclotetrasiloxane	556-67-2	R: **62**
(RS)-S-sec-butyl-O-ethyl-2-oxo-1,3-thiazolidin-3-ylphosphonothioate, fosthiazate (ISO) -	98886-44-3	R: **64**
Fentin acetate (ISO), triphenyltin acetate	900-95-8	R: **63**
Fentin hydroxide (ISO), triphenyltin hydroxide	76-87-9	R: **63**
Benz[a]pyrene, benzo[def]chrysene	50-32-8	R: **60-61**
n-Hexane	110-54-3	R: **62**
1,2-dibromo-3-chloropropane	96-12-8	R: **60**
Dodecachloropentacyclo[5.2.1.0<{POW}>2,6<{/POW}>.0<{POW}>3,9<{/POW}>.0<{POW}>5,8<{/POW}>] decane; mirex	2385-85-5	R: **62-63-64**
Diphenyl ether, pentabromo derivative, Pentabromodiphenyl ether	32534-81-9	R: **64**
2-bromopropane	75-26-3	R: **60**
2,3-dibromo-1-propanol, 2,3-dibromopropan-1-ol	96-13-9	R: **62**
2-methoxyethanol, ethylene glycol monomethyl ether	109-86-4	R**: 60-61**
2-ethoxyethanol, ethylene glycol monoethyl ether	110-80-5	R: **60-61**
Allyl 2,3-epoxypropyl ether, Allyl glycidyl ether, prop-2-en-1-yl 2,3-epoxypropyl ether	106-92-3	R: **62**
2,3-epoxypropan-1-ol, glycidol	556-52-5	R: **60**
2,4'-dichloro-(pyrimidin-5-yl)benzhydryl alcohol, fenarimol (ISO)	60168-88-9	R: **62-63-64**
2-methoxypropanol	1589-47-5	R: **61**
2-(2-methoxyethoxy)ethanol, diethylene glycol monomethyl ether	111-77-3	R: **63**
Bis(2-methoxyethyl) ether	111-96-6	R: **60-61**
R-2,3-epoxy-1-propanol	57044-25-4	R: **60**
2-(4-tert-butylphenyl)ethanol	5406-86-0	R: **62**
4,4-isobutylethylidenediphenol	6807-17-6	R: **60**
2-(2-hydroxy-3,5-dinitroanilino)ethanol	99610-72-7	R: **62**
Butyl methyl ketone, hexan-2-one, methyl butyl ketone, methyl-n-butyl ketone	591-78-6	R: **62**
6-methyl-1,3-dithiolo(4,5-b)quinoxalin-2-one, chinomethionat (ISO), quinomethionate	2439-01-2	R: **62**
2-methoxyethyl acetate, methylglycol acetate	110-49-6	R: **60-61**
2-ethoxyethyl acetate, ethylglycol acetate	111-15-9	R: **60-61**
Warfarin [1] (S)-4-hydroxy-3-(3-oxo-1-phenylbutyl)-2-benzopyrone [2] (R)-4-hydroxy-3-(3-oxo-1-phenylbutyl)-2-benzopyrone [3]	81-81-2 [1] 5543-57-7 [2] 5543-58-8 [3]	R: **61**
2-ethylhexyl[[[3,5-bis(1,1-dimethylethyl)-4-hydroxyphenyl]methyl]thio]acetate	80387-97-9	R: **61**
Bis(2-methoxyethyl) phthalate	117-82-8	R: **61-62**
2-ethylhexanoic acid	149-57-5	R: **63**
2-methoxypropyl acetate	70657-70-4	R: **61**

Chemical name	CAS number	Classification
Butyl (RS)-2-[4-(5-trifluoromethyl-2-pyridyloxy)phenoxy]propionate, fluazifop-butyl (ISO)	69806-50-4	R: **61**
N-3,5-dichlorophenyl-5-methyl-5-vinyl-1,3-oxazolidine-2,4-dione, vinclozolin (ISO)	50471-44-8	R: **60-61**
Methoxyacetic acid	625-45-6	R: **60-61**
DEHP, bis(2-ethylhexyl) phthalate, di-(2-ethylhexyl) phthalate	117-81-7	R**: 60-61**
DBP, dibutyl phthalate	84-74-2	R: **61-62**
(S)-2,3-dihydro-1H-indole-2-carboxylic acid	79815-20-6	R: **62**
(+/-) tetrahydrofurfuryl (R)-2-[4-(6-chloroquinoxalin-2-yloxy)phenyloxy]propionate	119738-06-6	R: **61**
3,5-dibromo-4-hydroxybenzonitrile, bromoxynil (ISO)	1689-84-5	R: **63**
4-hydroxy-3,5-diiodobenzonitrile, ioxynil (ISO)	1689-83-4	R: **63**
2,6-dibromo-4-cyanophenyl octanoate , bromoxynil octanoate (ISO)	1689-99-2	R: **63**
4-cyano-2,6-diiodophenyl octanoate, ioxynil octanoate (ISO)	3861-47-0	R: **63**
Nitrobenzene	98-95-3	R: **62**
2-sec-butyl-4,6-dinitrophenyl-3-methylcrotonate, binapacryl (ISO)	485-31-4	R**: 61**
6-sec-butyl-2,4-dinitrophenol, dinoseb	88-85-7	R**: 61-62**
2-tert-butyl-4,6-dinitrophenol, dinoterb (ISO)	1420-07-1	R: **61**
2,4-dichlorophenyl 4-nitrophenyl ether, nitrofen (ISO)	1836-75-5	R: **61**
2,6-dinitrotoluene	606-20-2	R: **62**
2,3-dinitrotoluene	602-01-7	R: **62**
3,4-dinitrotoluene	610-39-9	R: **62**
3,5-dinitrotoluene	618-85-9	R: **62**
2,5-dinitrotoluene	619-15-8	R: **62**
Methyl azoxy methyl acetate, methyl-ONN-azoxymethyl acetate	592-62-1	R: **61**
Disodium 4-amino-3-[[4'-[(2,4-diaminophenyl)azo][1,1'-biphenyl]-4-yl]azo]-5-hydroxy-6-(phenylazo)naphtalene-2,7-disulphonate; C.I. Direct Black 38	1937-37-7	R: **63**
Tetrasodium 3,3'-[[1,1'-biphenyl]-4,4'-diylbis(azo)]bis[5-amino-4-hydroxynaphthalene-2,7-disulphonate]; C.I. Direct Blue 6	2602-46-2	R: **63**
Disodium 3,3'-[[1,1'-biphenyl]-4,4'-diylbis(azo)]bis(4-aminonaphthalene-1-sulphonate); C.I. Direct Red 28	573-58-0	R: **63**
Thiocarbamide, thiourea	62-56-6	R: **63**
1,3-diphenylguanidine	102-06-7	R: **62**
2,6-dimethyl-4-tridecylmorpholine, tridemorph (ISO)	24602-86-6	R: **61**
2-imidazoline-2-thiol, ethylene thiourea, imidazolidine-2-thione	96-45-7	R: **61**
Propylenethiourea	2122-19-2	R: **63**
1,2,4-triazole	288-88-0	R: **63**
2-(4-chlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)hexanenitrile, myclobutanil	88671-89-0	R: **63**
Cycloheximide	66-81-9	R**: 61**
N-(7-fluoro-3,4-dihydro-3-oxo-4-prop-2-ynyl-2H-1,4-benzoxazin-6-yl)cyclohex-1-ene-1,2-dicarboxamide , flumioxazin (ISO)	103361-09-7	R: **61**
5-chloro-1,3-dihydro-2H-indol-2-one	17630-75-0	R: **62**
(2RS,3RS)-3-(2-chlorophenyl)-2-(4-fluorophenyl)-[(1H-1,2,4-triazol-1-yl)methyl]oxirane	106325-08-0	R**: 61**
N,N-dimethylformamide, dimethyl formamide	68-12-2	R: **61**
Acrylamide, prop-2-enamide	79-06-1	R: **62**

Chemical name	CAS number	Classification
N,N-dimethylacetamide	127-19-5	R: **61**
2-chloracetamide	79-07-2	R: **62**
Formamide	75-12-7	R: **61**
N-methylacetamide	79-16-3	R: **61**
N-methylformamide	123-39-7	R: **61**
(2RS,3RS;2RS,3SR)-2-(4-chlorophenyl)-3-cyclopropyl-1-(1H-1,2,4-triazol-1-yl)butan-2-ol, cyproconazole (ISO)	94361-06-5	R: **63**
3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea, linuron (ISO) E	330-55-2	R: **61-62**
Toluene	108-88-3	R: **63**
Nonylphenol	25154-52-3	R: **62-63**
4-nonylphenol, branched	84852-15-3	R: **62-63**
1-bromopropane, n-propyl bromide, propyl bromide	106-94-5	R: **60-63**
Lindane, γ-1,2,3,4,5,6-hexachlorocyclohexane, γ-HCH or γ-BHC	58-89-9	R: **64**
1,2,3-trichloropropane D	96-18-4	R: **60**
p-chlorobenzotrichloride, α,α,α,4-tetrachlorotoluene E	5216-25-1	R: **62**
Diphenylether; octabromo derivate	32536-52-0	R**: 61-62**
Malachite green hydrochloride (A) [1] Malachite green oxalate (B) [2]	569-64-2 [1] 18015-76-4 [2]	R: **63**
1,2-dimethoxyethane, EGDME, ethylene glycol dimethyl ether	110-71-4	R: **60-61**
1,2-Bis(2-methoxyethoxy)ethane, TEGDME, Triethylene glycol dimethyl ether, Triglyme	112-49-2	R: **61-62**
4,4'-isopropylidenediphenol, bisphenol A	80-05-7	R: **62**
1,3,5-trioxan, trioxymethylene	110-88-3	R: **63**
Bromoxynil heptanoate (ISO); 2,6-dibromo-4-cyanophenyl heptanoate	56634-95-8	R: **63**
BBP, benzyl butyl phtalate	85-68-7	R: **61-62**
4-nitrotoluene [1] 4-nitrotoluene [2] E	99-99-0	R: **62**
Dinocap (ISO) E	39300-45-3	R: **61**
2-nitrotoluene E	88-72-2	R: **62**
4,4'-oxydianiline [1] and its salts, p-aminophenyl ether E	101-80-4	R: **62**
1,2,4-triazol-3-ylamine, amitrole (ISO)	61-82-5	R: **63**
Carbendazim (ISO), methyl benzimidazol-2-ylcarbamate	10605-21-7	R: **60-61**
Benomyl (ISO), methyl 1-(butylcarbamoyl)benzimidazol-2-ylcarbamate	17804-35-2	R: **60-61**
S-ethyl 1-perhydroazepinecarbothioate, S-ethyl perhydroazepine-1-carbothioate, molinate (ISO)	2212-67-1	R: **62**
Cis-4-[3-(p-tert-butylphenyl)-2-methylpropyl]-2,6-dimethylmorpholine, fenpropimorph	67564-91-4	R: **63**
(2RS,3RS)-3-(2-chlorophenyl)-2-(4-fluorophenyl)-[(1H-1,2,4-triazol-1-yl)methyl]oxirane, epoxiconazol	133855-98-8	R: **62-63**
Methyl isocyanate	624-83-9	R: **63**
Isoxaflutole	141112-29-0	R: **63**
3-(3-chloro-p-tolyl)-1,1-dimethylurea, chlorotoluron	15545-48-9	R: **63**

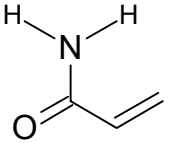
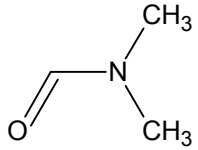
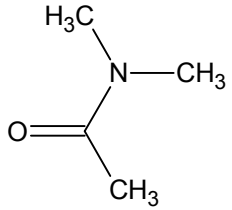
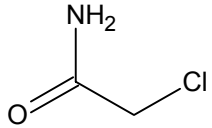
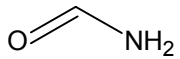
R60 (May impair fertility), R62 (Possible risk of impaired fertility
R61 (May cause harm to the unborn child),
R63 (Possible risk of harm to the unborn child.)
R64 (May cause harm to breastfed babies)

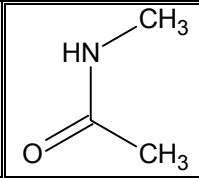
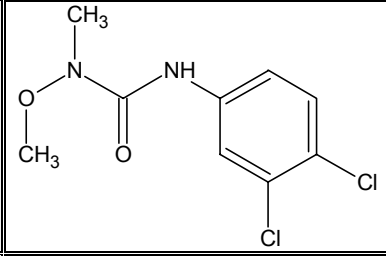
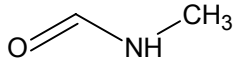
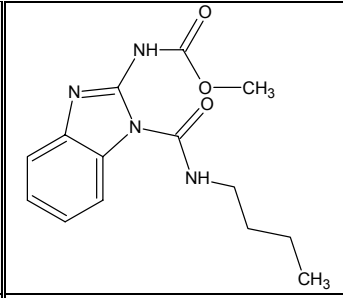
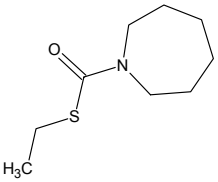
Appendix 2 Structure alerts present in DEREK

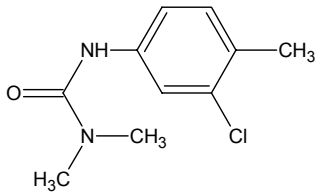
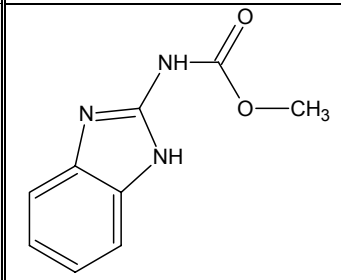
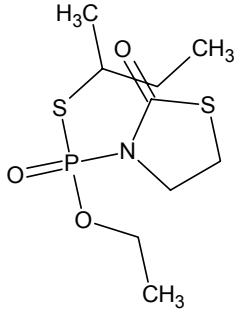
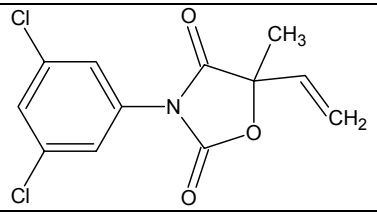
Toxicological end-point	Number of structure alerts present
Alpha-mu-globulin nephropathy	3
Anaphylaxis	1
Anticholinesterase activity	2
Bladder urothelial hyperplasia	1
Carcinogenicity	46
Cerebral oedema	1
Chloracne	3
Cumulative effects on white cell count type effect	1
Cyanide type effect	1
Developmental toxicity	3
Genotoxicity	1
Hepatotoxicity	2
High acute toxicity	4
Irritation of the eye and respiratory track	1
Irritation of eye	3
Irritation of gastrointestinal track	2
Irritation of respiratory track	2
Irritation of skin and eye	9
Irritation of skin, eye and respiratory track	16
Lachrymation	1
Methaemoglobinaemia	1
Mutagenicity	77
Neurotoxicity	6
Occupational asthma	1
Oestrogenicity	4
Peroxisome proliferation	7
Photoallergenicity	6
Pulmonar toxicity	1
Respiratory sensitization	13
Skin sensitization	61
Teratogenicity	5
Testicular toxicity	1
Thyroid toxicity	14
Uncoupler of oxidative phosphorylation	1

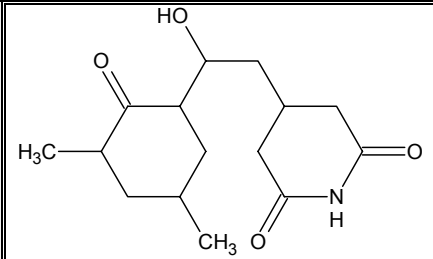
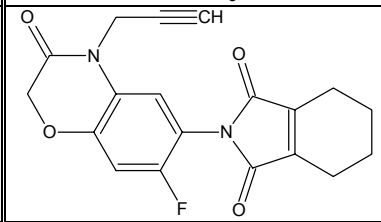
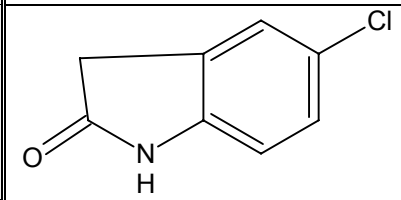
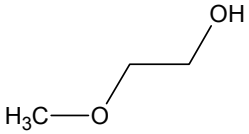
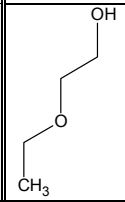
Appendix 3 DEREKfW predictions

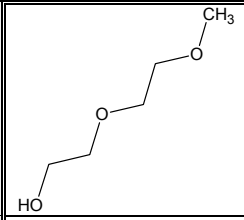
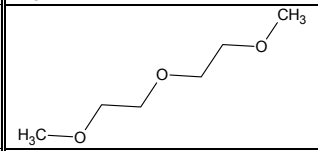
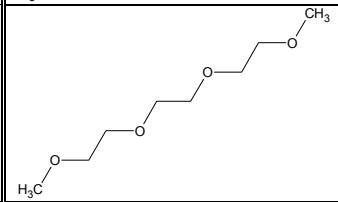
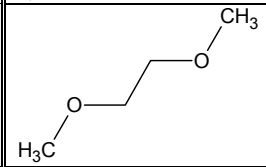
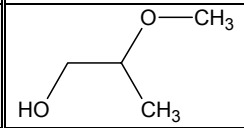
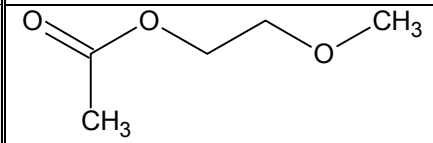
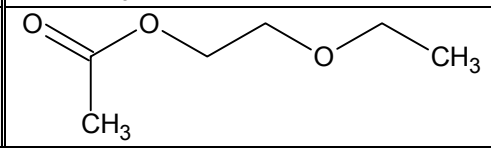
Predictions of DEREKfW compared to substances classified for reproductive toxicity according to Annex I and working database of the 29th ATP (according to Directive 67/548/EC).

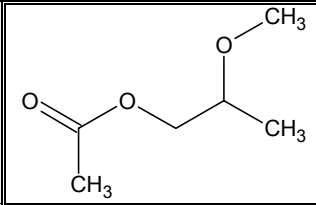
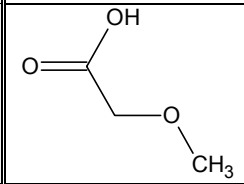
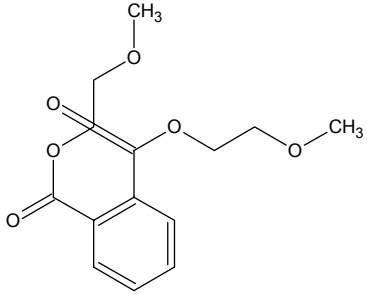
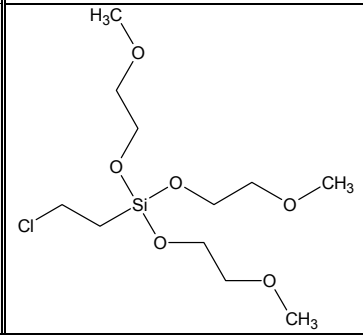
Substance identification:	Structure	Classification and labelling	Prediction DEREK	Effect DEREK
Chemical name: Acrylamide, prop-2-enamide CAS No.: 79-06-1		Fertility: R62 Developmental: - Lactation: -	Testicular: none Developmental: none Teratogenicity: none	**False negative**
Chemical name: N,N-dimethylformamide, dimethyl formamide CAS No.: 68-12-2		Fertility: - Developmental: R61 Lactation: -	Testicular: none Developmental: none Teratogenicity: none	**False negative**
Chemical name: N,N-dimethylacetamide CAS No.: 127-19-5		Fertility: - Developmental: R61 Lactation: -	Testicular: none Developmental: none Teratogenicity: none	**False negative**
Chemical name: 2-chloracetamide CAS No.: 79-07-2		Fertility: R62 Developmental: - Lactation: -	Testicular: none Developmental: none Teratogenicity: none	**False negative**
Chemical name: Formamide CAS No.: 75-12-7		Fertility: - Developmental: R61 Lactation: -	Testicular: none Developmental: none Teratogenicity: none	**False negative**

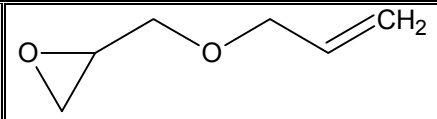
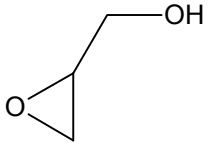
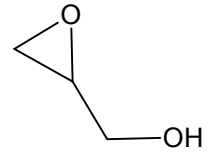
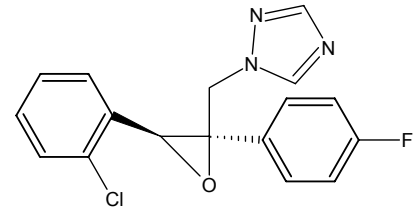
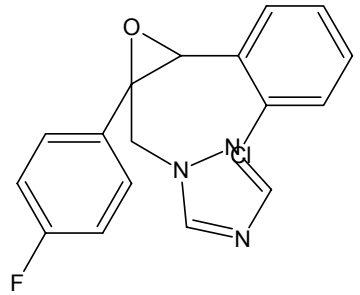
Substance identification:	Structure	Classification and labelling	Prediction DEREK	Effect DEREK
Chemical name: N-methylacetamide CAS No.: 79-16-3		Fertility: - Developmental: R61 Lactation: -	Testicular: none Developmental: none Teratogenicity: none	**False negative**
Chemical name: 3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea, linuron (ISO) CAS. No.: 330-55-2		Fertility: R62 Developmental: R61 Lactation: -	Testicular: none Developmental: none Teratogenicity: none	**False negative** **False negative**
Chemical name: N-methylformamide CAS No.: 123-39-7		Fertility: - Developmental: R61 Lactation: -	Testicular: none Developmental: none Teratogenicity: none	**False negative**
Chemical name: Benomyl (ISO), methyl 1-(butylcarbamoyl)benzimidazol-2-ylcarbamate CAS No.: 17804-35-2		Fertility: R60 Developmental: R61 Lactation: -	Testicular: none Developmental: none Teratogenicity: none	**False negative** **False negative**
Chemical name: S-ethyl 1-perhydroazepinecarbothioate, S-ethyl perhydroazepine-1-carbothioate, molinate (ISO) CAS No.: 2212-67-1		Fertility: R62 Developmental: Lactation: -	Testicular: none Developmental: none Teratogenicity: none	**False negative**

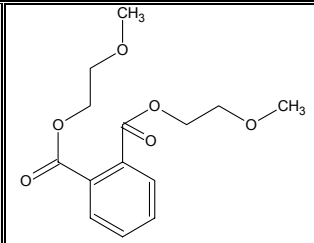
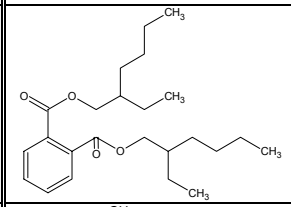
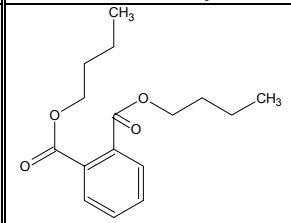
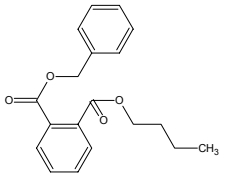
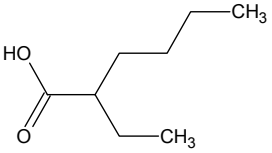
Substance identification:	Structure	Classification and labelling	Prediction DEREK	Effect DEREK
Chemical name: 3-(3-chloro-p-tolyl)-1,1-dimethylurea, chlorotoluron CAS No.: 15545-48-9		Fertility: Developmental: R63 Lactation: -	Testicular: none Developmental: none Teratogenicity: none	**False negative**
Chemical name: Carbendazim (ISO), methyl benzimidazol-2-ylcarbamate CAS No.: 10605-21-7		Fertility: R60 Developmental: R61 Lactation: -	Testicular: none Developmental: none Teratogenicity: none	**False negative** **False negative**
Chemical name: (RS)-S-sec-butyl-O-ethyl-2-oxo-1,3-thiazolidin-3-ylphosphonothioate, fosthiazate (ISO) CAS No.: 98886-44-3		Fertility: - Developmental: - Lactation: R64	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: N-3,5-dichlorophenyl-5-methyl-5-vinyl-1,3-oxazolidine-2,4-dione, vinclozolin (ISO) CAS No.: 50471-44-8		Fertility: R60 Developmental: R61 Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative** **False negative**

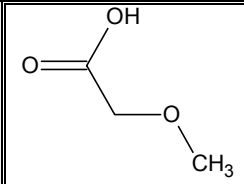
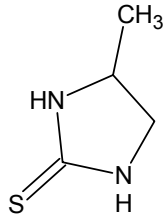
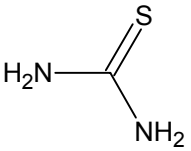
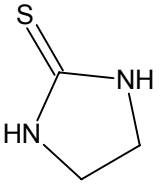
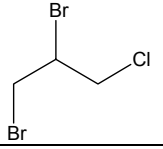
Substance identification:	Structure	Classification and labelling	Prediction DEREK	Effect DEREK
Chemical name: Cycloheximide CAS No.: 66-81-9		Fertility: Developmental: R61 Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: N-(7-fluoro-3,4-dihydro-3-oxo-4-prop-2-ynyl-2H-1,4-benzoxazin-6-yl)cyclohex-1-ene-1,2-dicarboxamide , flumioxazin (ISO) CAS No.: 103361-09-7		Fertility: Developmental: R61 Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: 5-chloro-1,3-dihydro-2H-indol-2-one CAS No.: 17630-75-0		Fertility: R62 Developmental: Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: 2-methoxyethanol, ethylene glycol monomethyl ether CAS No.: 109-86-4		Fertility: R60 Developmental: R61 Lactation: -	Testicular: plausible Developmental: plausible Teratogenicity: none	**Correct** **Correct**
Chemical name: 2-ethoxyethanol, ethylene glycol monoethyl ether CAS No.: 110-80-5		Fertility: R60 Developmental: R61 Lactation: -	Testicular: plausible Developmental: plausible Teratogenicity: none	**Correct** **Correct**

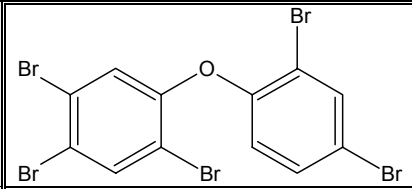
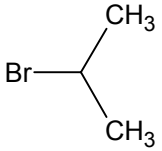
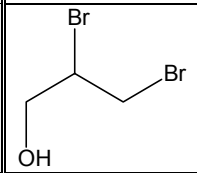
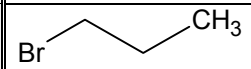
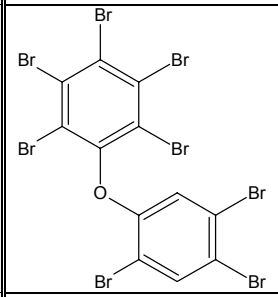
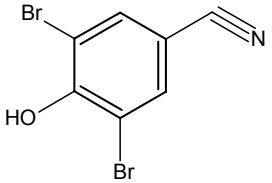
Substance identification:	Structure	Classification and labelling	Prediction DEREK	Effect DEREK
Chemical name: 2-(2-methoxyethoxy)ethanol, diethylene glycol monomethyl ether CAS No.: 111-77-3		Fertility: - Developmental: R63 Lactation: -	Testicular: none Developmental: none Teratogenicity: none	**False negative**
Chemical name: bis(2-methoxyethyl) ether CAS No.: 111-96-6		Fertility: R60 Developmental: R61 Lactation: -	Testicular: none Developmental: none Teratogenicity: none	**False negative** **False negative**
Chemical name: 1,2-Bis(2-methoxyethoxy)ethane, TEGDME, Triethylene glycol dimethyl ether, Triglyme CAS No.: 112-49-2		Fertility: R62 Developmental: R61 Lactation: -	Testicular: none Developmental: none Teratogenicity: none	**False negative** **False negative**
Chemical name: 1,2-dimethoxyethane, EGDME, ethylene glycol dimethyl ether CAS No.: 110-71-4		Fertility: R60 Developmental: R61 Lactation: -	Testicular: none Developmental: none Teratogenicity: none	**False negative** **False negative**
Chemical name: 2-methoxypropanol CAS No.: 1589-47-5		Fertility: - Developmental: R61 Lactation: -	Testicular: none Developmental: plausible Teratogenicity: none	**Correct**
Chemical name: 2-methoxyethyl acetate, methylglycol acetate CAS No.: 110-49-6		Fertility: R60 Developmental: R61 Lactation: -	Testicular: plausible Developmental: plausible Teratogenicity: none	**Correct** **Correct**
Chemical name: 2-ethoxyethyl acetate, ethylglycol acetate CAS No.: 111-15-9		Fertility: R60 Developmental: R61 Lactation: -	Testicular: plausible Developmental: plausible Teratogenicity: none	**Correct** **Correct**

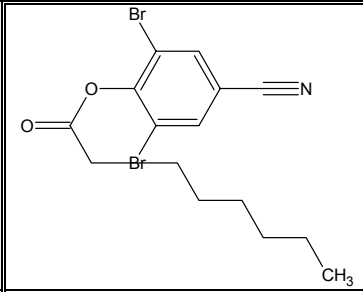
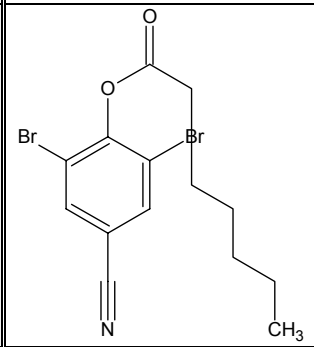
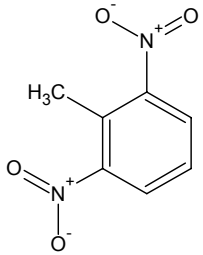
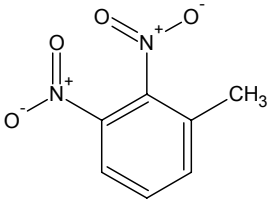
Substance identification:	Structure	Classification and labelling	Prediction DEREK	Effect DEREK
Chemical name: 2-methoxypropyl acetate CAS No.: 70657-70-4		Fertility: - Developmental: R61 Lactation: -	Testicular: none Developmental: plausible Teratogenicity: none	**Correct**
Chemical name: Methoxyacetic acid CAS No.: 625-45-6		Fertility: R60 Developmental: R61 Lactation: -	Testicular: plausible Developmental: plausible Teratogenicity: none	**Correct** **Correct**
Chemical name: bis(2-methoxyethyl) phthalate CAS No.: 117-82-8		Fertility: R62 Developmental: R61 Lactation: -	Testicular: plausible Developmental: plausible Teratogenicity: none Prediction for testicular and developmental toxicity based on the glycol ether	**Correct** **Correct**
Chemical name: 6-(2-chloroethyl)-6-(2-methoxyethoxy)-2,5,7,10-tetraoxa-6-silaundecane, etacelasil CAS No.: 37894-46-5		Fertility: - Developmental: R61 Lactation: -	Testicular: plausible Developmental: plausible Teratogenicity: none	**Correct**

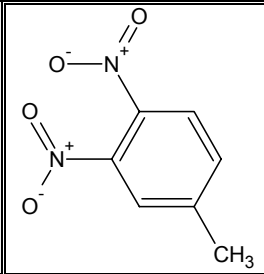
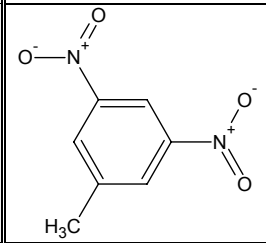
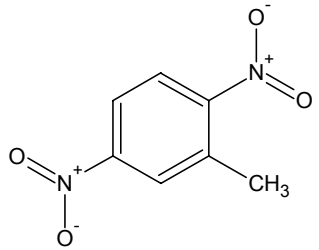
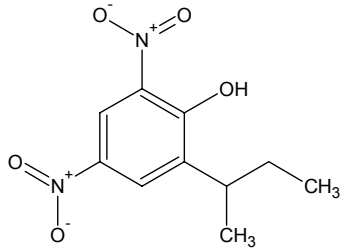
Substance identification:	Structure	Classification and labelling	Prediction DEREK	Effect DEREK
Chemical name: allyl 2,3-epoxypropyl ether, allyl glycidyl ether, prop-2-en-1-yl 2,3-epoxypropyl ether CAS No.: 106-92-3		Fertility: R62 Developmental: - Lactation: -	Testicular: none Developmental: none Teratogenicity: none	**False negative**
Chemical name: 2,3-epoxypropan-1-ol, glycidol CAS No.: 556-52-5		Fertility: R60 Developmental: - Lactation: -	Testicular: none Developmental: none Teratogenicity: none	**False negative**
Chemical name: R-2,3-epoxy-1-propanol CAS No.: 57044-25-4		Fertility: R60 Developmental: - Lactation: -	Testicular: none Developmental: none Teratogenicity: none	**False negative**
Chemical name: (2RS,3RS)-3-(2-chlorophenyl)-2-(4-fluorophenyl)-[(1H-1,2,4-triazol-1-yl)methyl]oxirane, epoxiconazol CAS No.: 133855-98-8		Fertility: R62 Developmental: R63 Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative** **False negative**
Chemical name: (2RS,3RS)-3-(2-chlorophenyl)-2-(4-fluorophenyl)-[(1H-1,2,4-triazol-1-yl)methyl]oxirane CAS No.: 106325-08-0		Fertility: Developmental: R61 Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative**

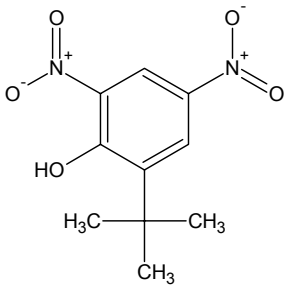
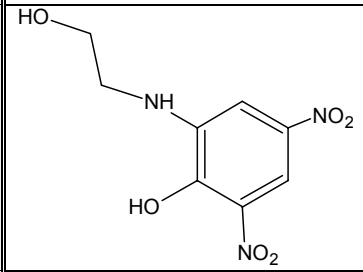
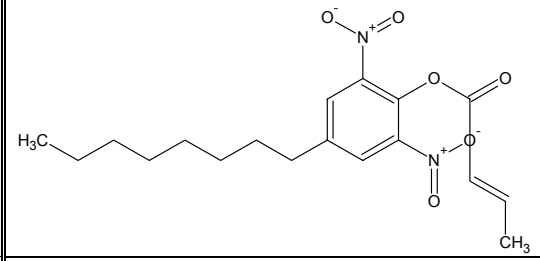
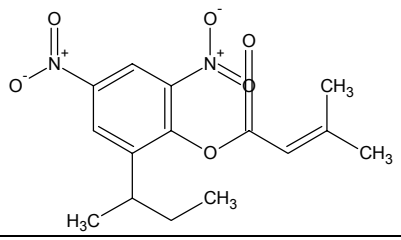
Substance identification:	Structure	Classification and labelling	Prediction DEREK	Effect DEREK
Chemical name: bis(2-methoxyethyl) phthalate CAS No.: 117-82-8		Fertility: R62 Developmental: R61 Lactation: -	Testicular: plausible Developmental: plausible Teratogenicity: none Prediction for testicular and developmental toxicity based on the glycol ether	**Correct** **Correct**
Chemical name: DEHP, bis(2-ethylhexyl) phthalate, di-(2-ethylhexyl) phthalate CAS No.: 117-81-7		Fertility: R60 Developmental: R61 Lactation: -	Testicular: none Developmental: none Teratogenicity: none	**False negative** **False negative**
Chemical name: DBP, dibutyl phthalate CAS No.: 84-74-2		Fertility: R62 Developmental: R61 Lactation: -	Testicular: none Developmental: none Teratogenicity: none	**False negative** **False negative**
Chemical name: BBP, benzyl butyl phtalate CAS No.: 85-68-7		Fertility: R62 Developmental: R61 Lactation: -	Testicular: none Developmental: none Teratogenicity: none	**False negative** **False negative**
Chemical name: 2-ethylhexanoic acid CAS No.: 149-57-5		Fertility: - Developmental: R63 Lactation: -	Testicular: none Teratogenicity: plausible Developmental: none	**Correct**

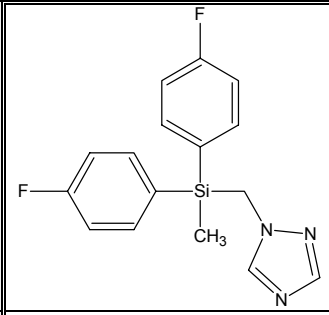
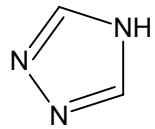
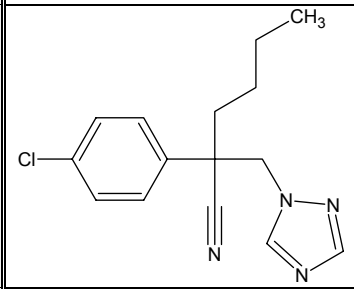
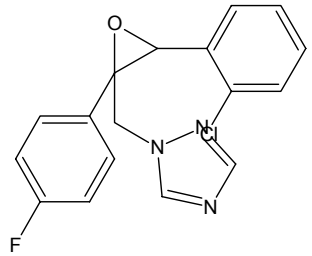
Substance identification:	Structure	Classification and labelling	Prediction DEREK	Effect DEREK
Chemical name: Methoxyacetic acid CAS No.: 625-45-6		Fertility: R60 Developmental: R61 Lactation: -	Testicular: plausible Developmental: plausible Teratogenicity: none Prediction for testicular and developmental toxicity based on the glycol ether	**Correct** **Correct**
Chemical name: propylenethiourea CAS No.: 2122-19-2		Fertility: - Developmental: R63 Lactation: -	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: Thiocarbamide, thiourea CAS No.: 62-56-6		Fertility: - Developmental: R63 Lactation: -	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: 2-imidazoline-2-thiol, ethylene thiourea, imidazolidine-2-thione CAS No.: 96-45-7		Fertility: - Developmental: R61 Lactation: -	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: 1,2-dibromo-3-chloropropane CAS No.: 96-12-8		Fertility: R60 Developmental: - Lactation: -	Testicular: none Teratogenicity: none Developmental: none	**False negative**

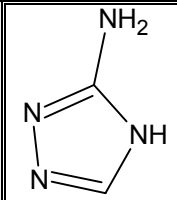
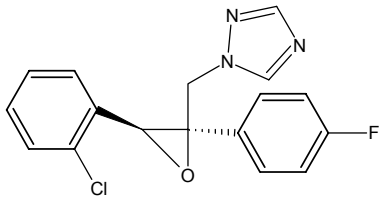
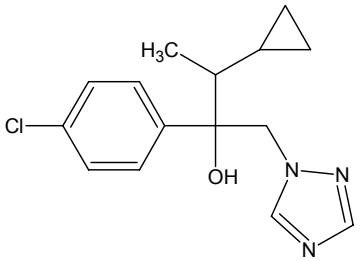
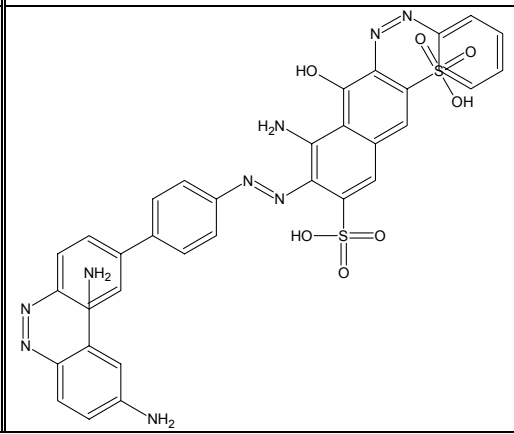
Substance identification:	Structure	Classification and labelling	Prediction DEREK	Effect DEREK
Chemical name: diphenyl ether, pentabromo derivative pentabromodiphenyl ether CAS No.: 32534-81-9		Fertility: - Developmental: - Lactation: R64	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: 2-bromopropane CAS No.: 75-26-3		Fertility: R60 Developmental: - Lactation: -	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: 2,3-dibromo-1-propanol, 2,3-dibromopropan-1-ol CAS No.: 96-13-9		Fertility: R62 Developmental: - Lactation: -	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: 1-bromopropane, n-propyl bromide, propyl bromide CAS No.: 106-94-5		Fertility: R60 Developmental: - Lactation: -	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: Diphenylether; octabromo derivate CAS No.: 32536-52-0		Fertility: R62 Developmental: R61 Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative** **False negative**
Chemical name: 3,5-dibromo-4-hydroxybenzonitrile, bromoxynil (ISO) CAS No.: 1689-84-5		Fertility: Developmental: R63 Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative**

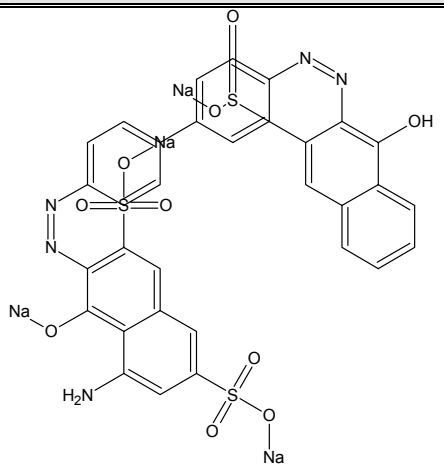
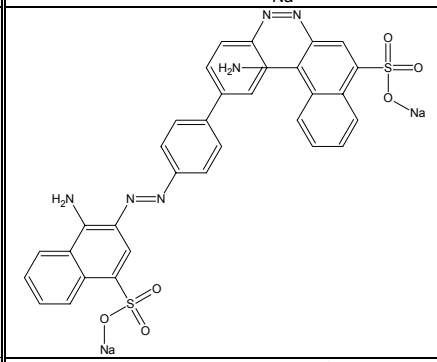
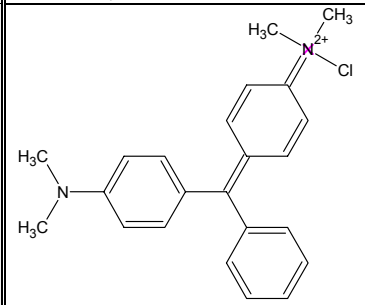
Substance identification:	Structure	Classification and labelling	Prediction DEREK	Effect DEREK
Chemical name: 2,6-dibromo-4-cyanophenyl octanoate , bromoxynil octanoate (ISO) CAS No.: 1689-99-2		Fertility: Developmental: R63 Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: bromoxynil heptanoate (ISO); 2,6-dibromo-4-cyanophenyl heptanoate CAS No.: 56634-95-8		Fertility: Developmental: R63 Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: 2,6-dinitrotoluene CAS No.: 606-20-2		Fertility: R62 Developmental: - Lactation: -	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: 2,3-dinitrotoluene CAS No.: 602-01-7		Fertility: R62 Developmental: - Lactation: -	Testicular: none Teratogenicity: none Developmental: none	**False negative**

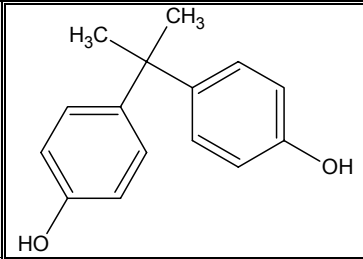
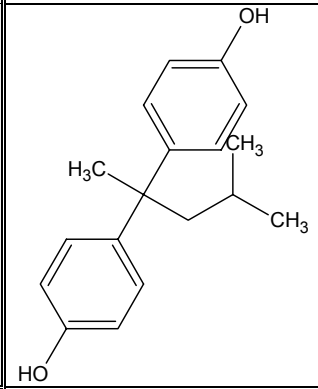
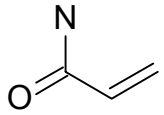
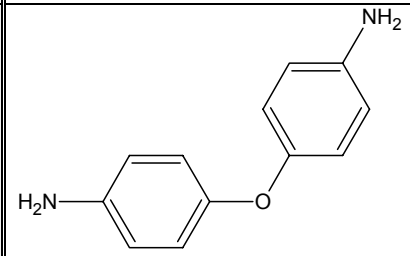
Substance identification:	Structure	Classification and labelling	Prediction DEREK	Effect DEREK
Chemical name: 3,4-dinitrotoluene CAS No.: 610-39-9		Fertility: R62 Developmental: - Lactation: -	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: 3.5-dinitrotoluene CAS No.: 618-85-9		Fertility: R62 Developmental: - Lactation: -	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: 2.5-dinitrotoluene CAS No.: 619-15-8		Fertility: R62 Developmental: - Lactation: -	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: 6-sec-butyl-2,4-dinitrophenol, dinoseb CAS No.: 88-85-7		Fertility: R62 Developmental: R61 Lactation: -	Testicular: none Teratogenicity: none Developmental: none	**False negative** **False negative**

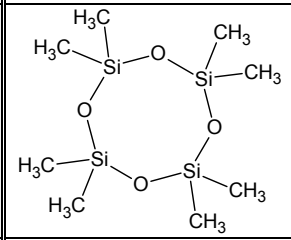
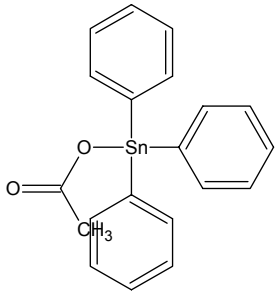
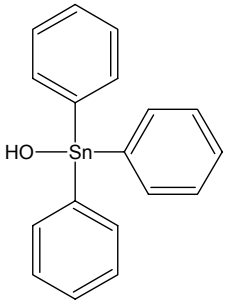
Substance identification:	Structure	Classification and labelling	Prediction DEREK	Effect DEREK
Chemical name: 2-tert-butyl-4,6-dinitrophenol, dinoterb (ISO) CAS No.: 1420-07-1		Fertility: - Developmental: R61 Lactation: -	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: 2-(2-hydroxy-3,5-dinitroanilino)ethanol CAS No.: 99610-72-7		Fertility: R62 Developmental: Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: Dinocap (iso) CAS No.: 39300-45-3		Fertility: Developmental: R61 Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: 2-sec-butyl-4,6-dinitrophenyl-3-methylcrotonate, binapacryl (ISO) CAS No.: 485-31-4		Fertility: Developmental: R61 Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative**

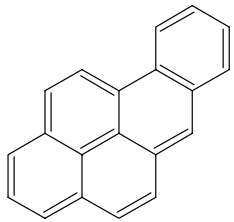
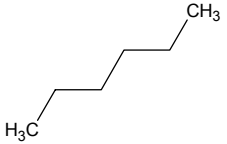
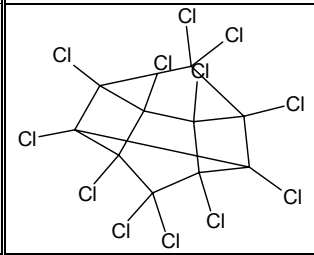
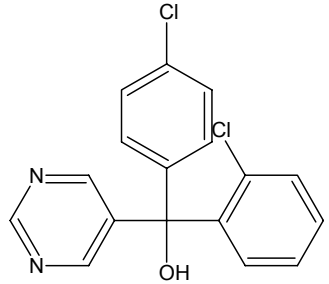
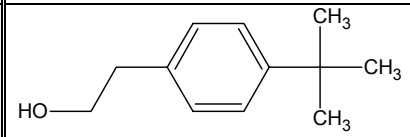
Substance identification:	Structure	Classification and labelling	Prediction DEREK	Effect DEREK
Chemical name: bis(4-fluorophenyl)(methyl)(1H-1,2,4-triazol-1-ylmethyl)silane, flusilazole (ISO) CAS No.: 85509-19-9		Fertility: Developmental: R61 Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: 1,2,4-triazole CAS No.: 288-88-0		Fertility: Developmental: R63 Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: 2-(4-chlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)hexanenitrile, myclobutanil CAS No.: 88671-89-0		Fertility: Developmental: R63 Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: (2RS,3RS)-3-(2-chlorophenyl)-2-(4-fluorophenyl)-[(1H-1,2,4-triazol-1-yl)methyl]oxirane CAS No.: 106325-08-0		Fertility: Developmental: R61 Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative**

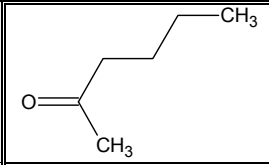
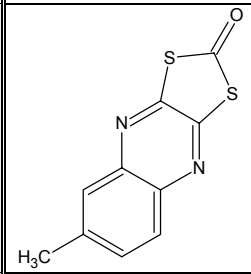
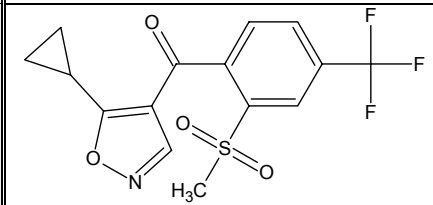
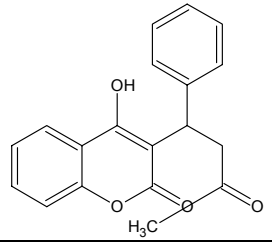
Substance identification:	Structure	Classification and labelling	Prediction DEREK	Effect DEREK
Chemical name: 1,2,4-triazol-3-ylamine, amitrole (ISO) CAS No.: 61-82-5		Fertility: Developmental: R63 Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: (2RS,3RS)-3-(2-chlorophenyl)-2-(4-fluorophenyl)-[(1H-1,2,4-triazol-1-yl)methyl]oxirane, epoxiconazol CAS No.: 133855-98-8		Fertility: R62 Developmental: R63 Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative** **False negative**
Chemical name: (2RS,3RS;2RS,3SR)-2-(4-chlorophenyl)-3-cyclopropyl-1-(1H-1,2,4-triazol-1-yl)butan-2-ol, cyproconazole (ISO) CAS No.: 94361-06-5		Fertility: Developmental: R63 Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: disodium 4-amino-3-[[4'-[(2,4-diaminophenyl)azo][1,1'-biphenyl]-4-yl]azo]-5-hydroxy-6-(phenylazo)naphtalene-2,7-disulphonate; C.I. Direct Black 38 CAS No.: 1937-37-7 DEREK training set substance		Fertility: Developmental: R63 Lactation:	Testicular: none Teratogenicity: none Developmental: plausible	**Correct**

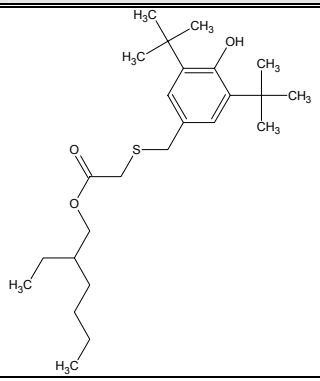
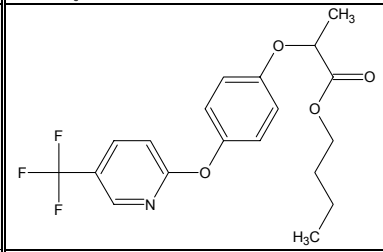
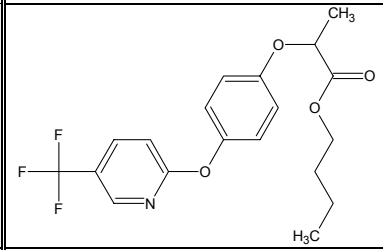
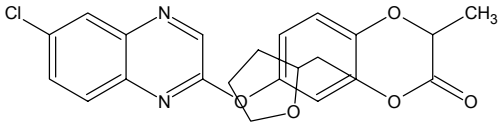
Substance identification:	Structure	Classification and labelling	Prediction DEREK	Effect DEREK
Chemical name: Tetrasodium 3,3'-[[1,1'-biphenyl]-4,4'-diylbis(azo)]bis[5-amino-4-hydroxynaphthalene-2,7-disulphonate]; C.I. Direct Blue 6 CAS No.: 2602-46-2 DEREK training set substance		Fertility: Developmental: R63 Lactation:	Testicular: none Teratogenicity: none Developmental: plausible	**Correct**
Chemical name: disodium 3,3'-[[1,1'-biphenyl]-4,4'-diylbis(azo)]bis(4-aminonaphthalene-1-sulphonate); C.I. Direct Red 28 CAS No.: 573-58-0 DEREK training set substance		Fertility: Developmental: R63 Lactation:	Testicular: none Teratogenicity: none Developmental: plausible	**Correct**
Chemical name: malachite green hydrochloride (A) [1] malachite green oxalate (B) [2] CAS No.: 569-64-2 [1] 18015-76-4 [2]		Fertility: Developmental: R63 Lactation:	Testicular: none Developmental: none Teratogenicity: none	**False negative**

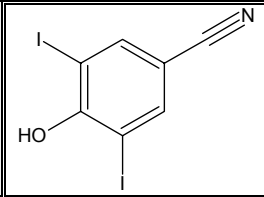
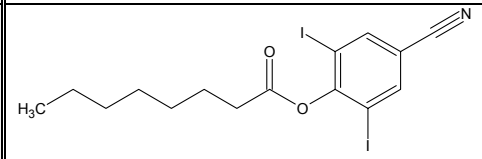
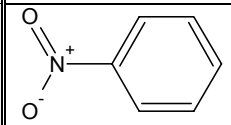
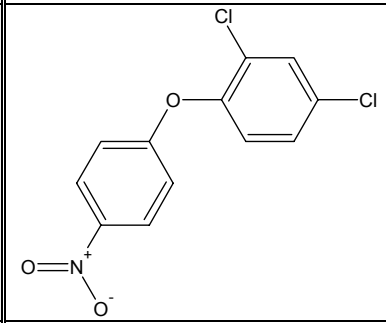
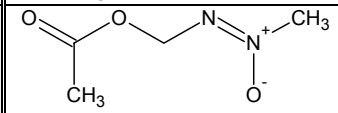
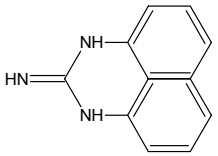
Substance identification:	Structure	Classification and labelling	Prediction DEREK	Effect DEREK
Chemical name: 4,4'-isopropylidenediphenol, bisphenol A CAS No.: 80-05-7		Fertility: R62 Developmental: Lactation:	Testicular: none Developmental: none Teratogenicity: none	**False negative**
Chemical name: 4,4-isobutylethylidenediphenol CAS No.: 6807-17-6		Fertility: R60 Developmental: Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: acrylamide, prop-2-enamide CAS No.: 79-06-1		Fertility: R62 Developmental: - Lactation: -	Testicular: none Developmental: none Teratogenicity: none	**False negative**
Chemical name: 4,4'-oxydianiline [1] and its salts, p-aminophenyl ether CAS No.: 101-80-4		Fertility: R62 Developmental: Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative**

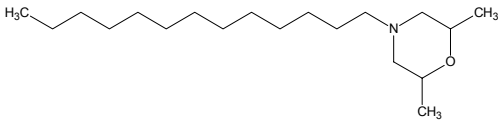
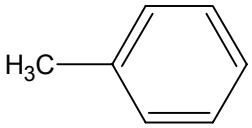
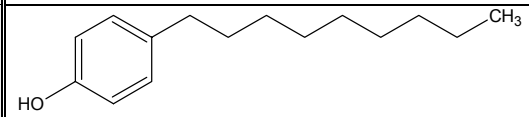
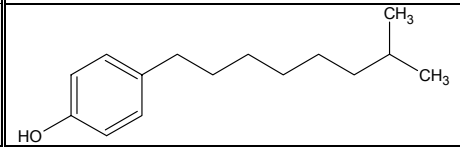
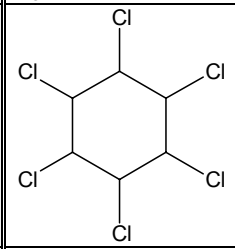
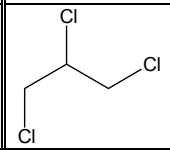
Substance identification:	Structure	Classification and labelling	Prediction DEREK	Effect DEREK
Chemical name: Carbon disulphide CAS No.: 75-15-0		Fertility: R62 Developmental: R63 Lactation: -	Testicular: none Teratogenicity: none Developmental: none	**False negative** **False negative**
Chemical name: octamethylcyclotetrasiloxane CAS No.: 556-67-2		Fertility: R62 Developmental: - Lactation: -	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: fentin acetate (ISO), triphenyltin acetate CAS No.: 900-95-8		Fertility: - Developmental: R63 Lactation: -	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: fentin hydroxide (ISO), triphenyltin hydroxide CAS No.: 76-87-9		Fertility: - Developmental: R63 Lactation: -	Testicular: none Teratogenicity: none Developmental: none	**False negative**

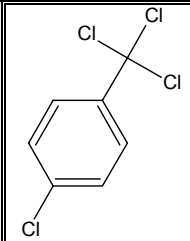
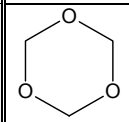
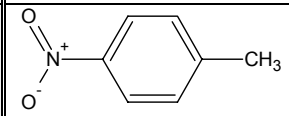
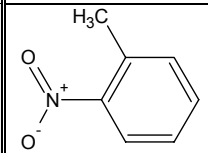
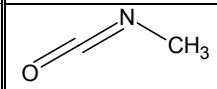
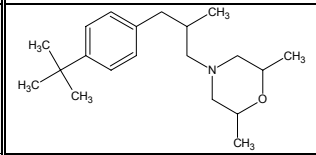
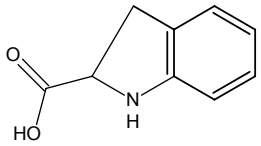
Substance identification:	Structure	Classification and labelling	Prediction DEREK	Effect DEREK
Chemical name: benz[a]pyrene, benzo[def]chrysene CAS No.: 50-32-8		Fertility: R60 Developmental: R61 Lactation: -	Testicular: none Teratogenicity: none Developmental: none	**False negative** **False negative**
Chemical name: n-hexane CAS No.: 110-54-3		Fertility: R62 Developmental: - Lactation: -	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: dodecachloropentacyclo[5.2.1.0<{POW}>2,6<{/POW}>.0<{POW}>3,9<{/POW}>.0<{POW}>5,8<{/POW}>]decane; mirex CAS No.: 2385-85-5		Fertility: R62 Developmental: R63 Lactation: -	Testicular: none Teratogenicity: none Developmental: none	**False negative** **False negative**
Chemical name: 2,4'-dichloro-¿-(pyrimidin-5-yl)benzhydryl alcohol, fenarimol (ISO) CAS No.: 60168-88-9		Fertility: R62 Developmental: R63 Lactation: R64	Testicular: none Teratogenicity: none Developmental: none	**False negative** **False negative** **False negative**
Chemical name: 2-(4-tert-butylphenyl)ethanol CAS No.: 5406-86-0		Fertility: R62 Developmental: Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative**

Substance identification:	Structure	Classification and labelling	Prediction DEREK	Effect DEREK
Chemical name: butyl methyl ketone, hexan-2-one, methyl butyl ketone, methyl-n-butyl ketone CAS No.: 591-78-6		Fertility: R62 Developmental: Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: 6-methyl-1,3-dithiolo(4,5-b)quinoxalin-2-one, chinomethionat (ISO), quinomethionate CAS No.: 2439-01-2		Fertility: R62 Developmental: Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: 5-cyclopropyl-1,2-oxazol-4-yl ¿,¿,¿-trifluoro-2-mesyl-p-tolyl ketone, isoxaflutole (ISO) CAS No.: 141112-29-0		Fertility: Developmental: R63 Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: warfarin [1] (S)-4-hydroxy-3-(3-oxo-1-phenylbutyl)-2-benzopyrone [2] (R)-4-hydroxy-3-(3-oxo-1-phenylbutyl)-2-benzopyrone [3] CAS No.: 81-81-2 5543-57-7 5543-58-8		Fertility: Developmental: R61 Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative**

Substance identification:	Structure	Classification and labelling	Prediction DEREK	Effect DEREK
Chemical name: 2-ethylhexyl[[[3,5-bis(1,1-dimethylethyl)-4-hydroxyphenyl]methyl]thio]acetate CAS No.: 80387-97-9		Fertility: Developmental: R61 Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: butyl (RS)-2-[4-(5-trifluoromethyl-2-pyridyloxy)phenoxy]propionate, fluazifop-butyl (ISO) CAS No.: 69806-50-4		Fertility: Developmental: R61 Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: butyl (RS)-2-[4-(5-trifluoromethyl-2-pyridyloxy)phenoxy]propionate, fluazifop-P-butyl (ISO) CAS No.: 79241-46-6		Fertility: Developmental: R63 Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: (+/-) tetrahydrofurfuryl (R)-2-[4-(6-chloroquinoxalin-2-yloxy)phenyloxy]propionate CAS No.: 119738-06-6		Fertility: R62 Developmental: R61 Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative** **False negative**

Substance identification:	Structure	Classification and labelling	Prediction DEREK	Effect DEREK
Chemical name: 4-hydroxy-3,5-diiodobenzonitrile, ioxynil (ISO) CAS No.: 1689-83-4		Fertility: Developmental: R63 Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: 4-cyano-2,6-diiodophenyl octanoate, ioxynil octanoate (ISO) CAS No.: 3861-47-0		Fertility: Developmental: R63 Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: Nitrobenzene CAS No.: 98-95-3		Fertility: R62 Developmental: Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: 2,4-dichlorophenyl 4-nitrophenyl ether, nitrofen (ISO) CAS No.: 1836-75-5		Fertility: Developmental: R61 Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: methyl azoxy methyl acetate, methyl-ONN-azoxymethyl acetate CAS No.: 592-62-1		Fertility: Developmental: R61 Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: 1,3-diphenylguanidine CAS No.: 102-06-7		Fertility: R62 Developmental: Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative**

Substance identification:	Structure	Classification and labelling	Prediction DEREK	Effect DEREK
Chemical name: 2,6-dimethyl-4-tridecylmorpholine, tridemorph (ISO) CAS No.: 24602-86-6		Fertility: Developmental: R61 Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: toluene CAS No.: 108-88-3		Fertility: Developmental: R63 Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: nonylphenol CAS No.: 25154-52-3		Fertility: Developmental: R63 Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: Nonylphenol, branched CAS No.: 84852-15-3		Fertility: Developmental: R63 Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: lindane, γ-1,2,3,4,5,6-hexachlorocyclohexane, γ-HCH or γ-BHC CAS No.: 58-89-9		Fertility: Developmental: Lactation: R64	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: 1,2,3-trichloropropane CAS No.: 96-18-4		Fertility: R60 Developmental: Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative**

Substance identification:	Structure	Classification and labelling	Prediction DEREK	Effect DEREK
Chemical name: p-chlorobenzotrichloride, α,α,α,4-tetrachlorotoluene CAS No.: 5216-25-1		Fertility: R62 Developmental: Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: 1,3,5-trioxan, trioxymethylene CAS No.: 110-88-3		Fertility: Developmental: R63 Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: 4-nitrotoluene [1] 4-nitrotoluene [2] CAS No.: 99-99-0		Fertility: R62 Developmental: Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: 2-nitrotoluene CAS No.: 88-72-2		Fertility: R62 Developmental: Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: Methyl isocyanate CAS No.: 624-83-9		Fertility: Developmental: R63 Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: cis-4-[3-(p-tert-butylphenyl)-2-methylpropyl]-2,6-dimethylmorpholine, fenpropimorph CAS No.: 67564-91-4		Fertility: Developmental: R63 Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative**
Chemical name: (S)-2,3-dihydro-1H-indole-2-carboxylic acid CAS No.: 79815-20-6		Fertility: R62 Developmental: Lactation:	Testicular: none Teratogenicity: none Developmental: none	**False negative**