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Applicability of the Threshold of Toxicological Concern (TTC) approach to cosmetics – preliminary analysis

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ABSTRACT

This report describes the application of chemoinformatic methods to explore the applicability of the Threshold of Toxicological Concern (TTC) approach to cosmetic ingredients. For non-cancer endpoints, the most widely used TTC approach is the Cramer classification scheme, which categorises chemicals into three classes (I, II and III) depending on their expected level of concern for oral systemic toxicity (low, medium, high, respectively). The chemical space of the Munro non-cancer dataset was characterised to assess whether this underlying TTC dataset is representative of the "world" of cosmetic ingredients, as represented by the COSMOS Cosmetics Inventory. In addition, the commonly used Cramer-related Munro threshold values were applied to a toxicological dataset of cosmetic ingredients, the COSMOS TTC dataset, to assess the degree of protectiveness resulting from the application of the Cramer classification scheme. This analysis is considered preliminary, since the COSMOS TTC dataset and Cosmetics Inventory are subject to an ongoing process of extension and quality control within the COSMOS project.

The results of this preliminary analysis show that the Munro dataset is broadly representative of the chemical space of cosmetics, although certain structural classes are missing, notably organometallics, silicon-containing compounds, and certain types of surfactants (non-ionic and cationic classes). Furthermore, compared with the Cosmetics Inventory, the Munro dataset has a higher prevalence of reactive chemicals and a lower prevalence of larger, long linear chain structures. The COSMOS TTC dataset, comprising repeat dose toxicity data for cosmetics ingredients, shows a good representation of the Cosmetics Inventory, both in terms of physicochemical property ranges, structural features and chemical use categories. Thus, this dataset is considered to be suitable for investigating the applicability of the TTC approach to cosmetics. The results of the toxicity data analysis revealed a number of cosmetic ingredients in Cramer Class I with No Observed Effect Level (NOEL) values lower than the Munro threshold of 3000 µg/kg bw/day. The prevalence of these "false negatives" was less than 5%, which is the percentage expected by chance resulting from the use of the 5th percentile of cumulative probability distribution of NOELs in the derivation of TTC values. Furthermore, the majority of these false negatives do not arise when structural alerts for DNA-binding are used to identify potential genotoxicants, to which a lower TTC value of 0.0025 µg/kg bw/day is typically applied. Based on these preliminary results, it is concluded that the current TTC approach is broadly applicable to cosmetics, although a number of improvements can be made, through the quality control of the underlying TTC datasets, modest revisions / extensions of the Cramer classification scheme, and the development of explicit guidance on how to apply the TTC approach.

LIST OF ABBREVIATIONS

COLIPA	European Cosmetics Association
DG SANCO	Directorate General for Health & Consumer Protection (European Commission)
EFSA	European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organization
FDA	United States Food and Drug Administration
HOMO	Highest Occupied Molecular Orbital
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JRC	Joint Research Centre (European Commission)
ILSI	International Life Sciences Institute
LUMO	Lowest Unoccupied Molecular Orbital
NOAEL	No observed adverse effect level
NOEL	No observed effect level
QSAR	Quantitative Structure-Activity Relationship
SA	Structural alert
SCCP	Scientific Committee on Consumer Products
SCCS	Scientific Committee on Consumer Safety
SCENIHR	Scientific Committee on Emerging and Newly Identified Health Risks
SCHER	Scientific Committee on Health and Environmental Risks
TTC	Threshold of Toxicological Concern
WHO	World Health Organization

CONTENTS

1. Scientific background

1.1 Introduction to the TTC concept

The Threshold of Toxicological Concern (TTC) concept refers to the establishment of a generic oral exposure level for (groups of) chemicals below which there is expected to be no appreciable risk to human health (Barlow, 2005). The TTC approach can be a useful screening or data-gap filling tool for chemicals for which substance-specific toxicity data are not available or routinely required in regulatory submissions (for example, metabolites and impurities), provided that reliable exposure data are also available.

Originally, the TTC approach was used in the assessment of indirect food additives (contact substances) and food flavourings. Subsequently, the approach has been investigated and proposed for use in a wide range of regulatory areas, including the assessment of chemicals in consumer products, and in particular cosmetic ingredients and impurities (Blackburn et al, 2005; Kroes et al., 2007). The applicability of the approach to chemicals in food and feed safety areas has been evaluated by EFSA (EFSA, 2011), based on the work of an EFSA working group (referred to hereafter as the EFSA TTC WG).

1.2 Cramer decision tree

In the application of the TTC concept to non-cancer endpoints, the Cramer decision tree is probably the most commonly used approach for classifying and ranking chemicals on the basis of their expected level of oral toxicity. It was proposed by Cramer, Ford and Hall in 1978 (Cramer et al, 1978) as a priority setting tool in the safety assessment of food additives which would make expert judgements more transparent, explicit and rational, and thus more reproducible and trustworthy. The scheme was derived from the authors' earlier experience in classifying food flavours (Oser & Hall, 1977) and their subsequent work in evaluating a range of carcinogens, pesticides and industrial chemicals (Cramer et al, 1978).

The original Cramer decision tree consists of 33 questions, each answered "yes" or "no" and leading to another question or to the final classification into one of the three classes (I, II and III) as follows:

Class I Substances with simple chemical structures and for which efficient modes of metabolism exist, suggesting a low order of oral toxicity.

Class II Substances which possess structures that are less innocuous than class I substances, but do not contain structural features suggestive of toxicity like those substances in class III.

Class III Substances with chemical structures that permit no strong initial presumption of safety or may even suggest significant toxicity or have reactive functional groups.

The logic of the sequential questions was based on the then available knowledge on toxicity and on how chemical structures were metabolised in mammalian metabolic pathways. The questions relate mostly to chemical structure, but natural occurrence in the body and in food are also taken into consideration. The tree is intended for use with all ingested, structurally defined organic and metallo-organic substances.

The Cramer scheme was tested against 81 chemicals including pesticides, drugs, food additives and industrial chemicals with known no observed effect level (NOEL) values reported in terms of dietary concentrations in short-terms or chronic studies (Cramer et al. 1978). Although there was overlap in the range of magnitudes of the NOELs between the three structural classes, it was clear that the

NOELs of Class I substances were generally higher than those of Class III, with those of Class II being in between. Noteworthy, there was no underestimation of toxicity when compared with the available chronic oral toxicity data.

To facilitate the consistent and transparent application of the TTC approach, including the assessment of both cancer and non-cancer endpoints, the JRC has developed the Toxtree software (http://ihcp.jrc.ec.europa.eu/our_labs/computational_toxicology/qsar_tools/toxtree), in collaboration with various partners, including IdeaConsult Ltd (Bulgaria), Curios-IT (The Netherlands) and the Istituto di Sanita' (Italy). The Toxtree implementation of the Cramer scheme has been evaluated by Patlewicz and coworkers (2008), and by Lapenna and Worth (2011).

1.3 Derivation of human exposure threshold values

The Cramer decision tree was subsequently used by Munro and coworkers with the purpose of deriving human exposure levels (TTC values) for toxicity endpoints other than carcinogenicity (Munro et al., 1996). The Munro dataset comprised over 613 organic chemicals with associated 2941 NOEL values derived from a variety of non-cancer endpoints from sub-chronic, chronic, reproductive and developmental toxicity studies carried out in rodents and rabbits. The authors assigned each chemical in the dataset to one of three classes based on the Cramer scheme. They also derived human exposure threshold values by taking the lower fifth percentile value of the distribution of NOELs for each Cramer class, multiplying this value by 60 to convert from mg/kg body weight per day into mg/person per day, and then dividing by a factor of 100 to ensure a margin of safety. On this basis, Munro and coworkers proposed TTC values of 1800, 540 and 90 µg/person/day (corresponding to 30, 9 and 1.5 µg/kg/day) for Cramer classes I, II and III, respectively.

In addition to the above-mentioned TTC levels for non-cancer endpoints, specific (and lower) TTC levels have also been derived for compounds with structural alerts for genotoxicity (0.15 µg/person/day; 0.0025 µg/kg.day) and for organophosphates (18 µg/person/day; 0.3 µg/kg.day) (Kroes et al., 2004), the general idea being that these lower threshold values should be applied in a tiered assessment approach before the Munro non-cancer threshold values.

The various TTC values are summarised in Table 1.1

Table 1.1. Commonly used TTC values

Type of threshold	TTC value µg/person per day	TTC value µg/kg bw per day
Structural alert for genotoxicity	0.15	0.0025
Structural alert for AchE inhibition (OPs and carbamates)	18	0.3
Cramer Class III	90	1.5
Cramer Class II	540	9.0
Cramer Class I	1800	30

The TTC levels proposed by Munro are now widely used in the food safety area, for example in the international evaluation of flavouring substances which was first applied by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1997 (WHO, 1997). However, it remains an open question whether these TTC levels are suitable in other areas of regulatory application, or whether alternative threshold values need to be derived from more extensive or application-specific dataset (as an extension or alternative to the Munro dataset). This is not just a scientific question, but also a matter for policy formulation.

2. Background to the study

2.1 The COSMOS project

The COSMOS (Integrated In silico Models for the Prediction of Human Repeated Dose Toxicity of COSMetics to Optimise Safety) Project[1] is jointly funded by the European Commission and the European Cosmetics Association (COLIPA). It is part of the SEURAT-1 Research Initiative[2], which is developing alternative (non-animal) methods to support the safety assessment of cosmetic ingredients. The SEURAT-1 projects, including COSMOS, started on 1 January 2011 and will run until 31 December 2015.

The overall aim of COSMOS is to develop an integrated suite of computational workflows that will allow for the prediction of repeat dose toxicity to humans through the integration of models based on the threshold of toxicological concern (TTC) approach, innovative chemistry such as quantitative structure-activity relationships (QSAR), and multi-scale modelling such as physiologically based pharmacokinetics (PBPK).

The specific objectives of COSMOS are to: a) collate and curate new sources of toxicological data; b) create an inventory of known cosmetic ingredients and their associated chemical structures; c) develop the TTC approach and assess its applicability to cosmetics; d) develop innovative toxicity prediction strategies based on chemical categories and QSAR related to key events in adverse outcome pathways; e) develop a multi-scale modelling approach to predict target organ concentrations and extrapolate from in vitro to in vivo exposure scenarios; and f) use the KNIME technology to integrate access to databases and modelling approaches into flexible computational workflows that will be made publicly accessible for use in the safety assessment of cosmetics and other chemicals in consumer products.

2.2 The European Commission Working Group on TTC

In November 2008, the Directorate General for Health & Consumer Protection (DG SANCO) of the European Commission (EC) released a preliminary report representing a "Draft Opinion on the Use of the Threshold of Toxicological Concern (TTC) Approach for Human Safety Assessment of Chemical Substances with focus on Cosmetics and Consumer Products" (SCHER/SCCP/SCENIHR, 2008). This was developed by a Working Group (referred to hereadter as the EC TTC WG) representing the three non-food Scientific Committees (SCs): the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR).

In accordance with its mandate[3], the EC TTC WG had been asked to evaluate the potential applications of the TTC approach for human health risk assessment of cosmetics and other consumer products in relation to the mandates of the three SCs.

A public consultation of the preliminary report took place from 24 November 2008 to 2 January 2009, and a targeted hearing with the stakeholders who contributed to the public consultation took place on 24 September 2009.

On 8 June 2011, DG SANCO organised a joint meeting of the EC TTC WG with the EFSA TTC WG, which had been developing in parallel an opinion on the applicability of the TTC apporach in the area of food and feed safety, to exchange on the status of the respective work carried out by the two WGs.

[1] http://www.cosmostox.eu/
[2] http://www.seurat-1.eu/
[3] http://ec.europa.eu/health/archive/ph_risk/documents/ttc_mandate.pdf

The project described in this report was initiated by the JRC as a follow-up to the joint meeting of the EC and EFSA TTC WGs, in order to explore the relevance of the Munro non-cancer dataset in deriving TTC values to cosmetics. The results were made available to DG SANCO to support the finalisation of the European Commission Working Group on TTC opinion on the applicability of the TTC approach to chemicals in consumer products

2.3 Aims of the study

The aims of this project were to apply chemoinformatic methods in order to assess:

a) whether is the underyling non-cancer TTC (Munro) dataset representative of the "world" of cosmetic ingredients, including hair dyes, UV filters, and "complex structures" with combination of functional groups;

b) the degree of protectiveness provided by the Cramer-related (Munro) threshold values for cosmetic ingredients.

3. Datasets and software tools

In this project, the **Munro TTC dataset** as well two preliminary datasets being developed within the COSMOS project were used: the **COSMOS non-cancer TTC dataset**, containing repeat-dose toxicity data for cosmetic ingredients; and the **COSMOS Cosmetics Inventory**, a compilation of substances from the EU CosIng and US Personal Care Products Council (PCPC) lists. These datasets are further described below.

3.1 The Munro TTC dataset

The Munro dataset is currently the *de facto* TTC database for non-cancer endpoints. The dataset is based on a 1996 publication (Munro et al, 1996) and contains 613 tested chemicals by name (607 unique CAS RNs). The structure data file and summary data tables are downloadable from EFSA (http://www.efsa.europa.eu/de/supporting/pub/159e.htm). The summarised data include study design, NOEL values and the associated critical effects. These files were compiled for the purposes of a chemoinformatics investigation carried out by Soluzioni Informatiche srl (Bassan et al, 2011) under the terms of an EFSA contract.

3.2 The COSMOS TTC dataset

The COSMOS TTC dataset was derived by matching cosmetics ingredients in the Cosmetics Inventory with oral repeat dose toxicity data from five toxicity data sources: the Munro dataset (Munro et al, 1996, RepDose[4], ToxRefDB[5], FDA PAFA[6], and ILSI DevTox[7]. The following criteria were used to select the NOEL values from various data sources:

- Oral repeated dose toxicity studies included subchronic, chronic, reproductive, developmental, multigeneration reproductive-developmental, immunology, and neurotoxicity. For target organ studies, rat, mouse, dog and monkey studies were used. For reproductive toxicity, developmental, rat, mouse and rabbit studies were used.

- In general, minimum NOEL values (such as ToxRefDB or FDA PAFA) were selected. However, NOAEL values from regulatory sources were used whenever available.

Version 1.0 of the COSMOS TTC dataset consists of 660 substances. After removal of chemicals with undefined structures, the dataset consisted of 558 substances with well defined structures. Within the COSMOS project, the dataset will be regularly updated in terms of its chemical coverage (adding chemicals from additional toxicity data sources) as well as the quality control of the chemical structures and toxicity data.

For the purposes of the Cramer classification and NOEL distribution analyses in this study, the dataset was further reduced to 385 substances with well-defined structures and qualified NOEL values (see Sections 5 and 6, below).

[4] http://www.fraunhofer-repdose.de/
[5] http://www.epa.gov/ncct/toxrefdb/
[6] http://www.fda.gov/food/foodingredientspackaging/ucm115326.htm
[7] http://www.ilsi.org/ResearchFoundation/Pages/DevelopmentalToxicityDatabase.aspx

3.3 The COSMOS Cosmetics Inventory

The Cosmetics Inventory contains lists from both the EU and the US: the EU CosIng list was downloaded in April 2011 from the European Commission's website (http://ec.europa.eu/consumers/cosmetics/cosing/). The US cosmetics list was obtained from the publication of the Personal Care Product Council (PCPC, 2011). The COSING inventory consists of 9286 unique CAS RNs and 19,390 unique INCI names. The PCPC inventory lists 3,716 unique CAS RNs and 3,657 unique INCI names.

The Venn Diagram in Figure 3.1 illustrates the overlap between the COSING and PCPC inventories by CAS RNs as well as Names. They clearly indicate that there are many-to-many relationships between the CAS RN and INCI name. The overlap was used to define the COSMOS Cosmetics Inventory. Version 1.0 of the Cosmetics Inventory consists of 4460 chemicals with well-defined structures that are found in CosIng and/or PCPC. Within the COSMOS project, the inventory will be updated in terms of its chemical coverage and inclusion of quality-controlled chemical structures.

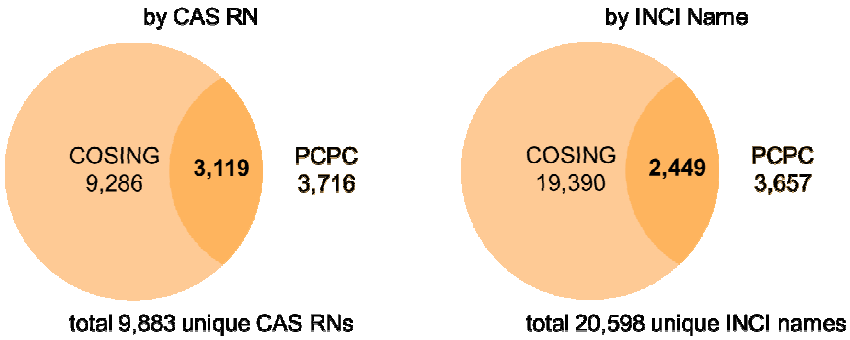

Figure 3.1. Sources of chemicals in theCOSMOS Cosmetics Inventory

3.4 Use categories in the COSMOS Cosmetics Inventory and TTC dataset

Since the COSMOS Non-Cancer TTC dataset is a subset of the entire Cosmetics Inventory, it is important to examine whether the subset retains the diversity and distributions of the full inventory. Figure 3.2 illustrates the compound distributions across use categories for the top 11 categories.

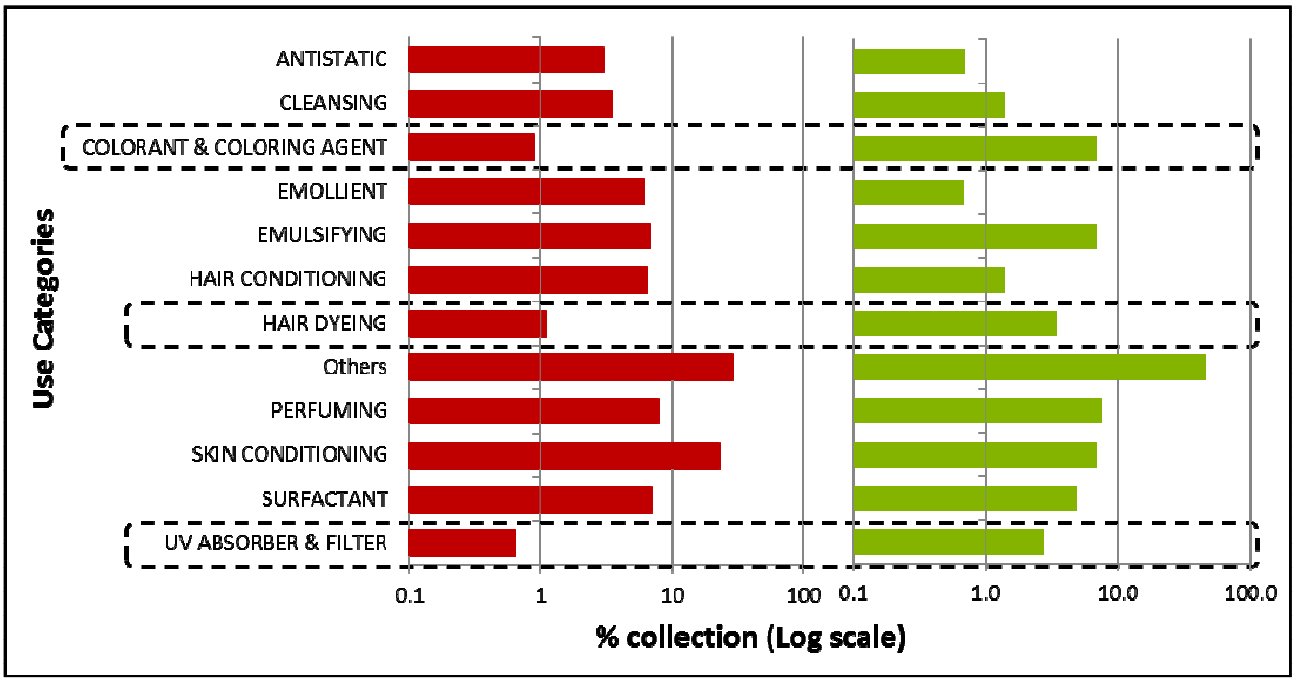

Figure 3.2. Comparison of the COSMOS TTC dataset and Cosmetics Inventory by use type (RED = Cosmetics Inventory; GREEN = COSMOS TTC)

Overall, the non-cancer TTC dataset retains a good representation of the Cosmetics Inventory, with 75% of the full list of inventory use categories being represented in the TTC subset. The distributions across the top 11 categories are very similar (Figure 3.2). However, there are some cases where the TTC subset has a smaller prevalence including skin conditioning, emollient, hair conditioning, and antistatic agents. On the other hand, the TTC subset had a higher prevalence of colourants/colouring agents, UV absorbers/filters, humectants and masking agents.

The category labelled “others” represents all other use categories including preservative (antioxidant, antimicrobial, etc.), skin care (skin protecting humectant, moisturising, tonic, astringent, tanning, etc.), solvent, oral care, plasticiser, flavouring, and hair care (antidandruff, hair fixing, hair waving or straightening, etc.).

4. Chemical space analysis

4.1 Definition of chemical space

Chemical space is a representation of the structural features and/or molecular properties covered by a defined set of chemicals. The molecular properties may include intrinsic properties (defined purely by chemical structure), such as size and shape, derived properties such as chemical reactivity, as well as extrinsic and biologically relevant properties such as metabolic activity.

By using chemoinformatic methods, it is possible to visualise and characterise chemical space in a consistent manner, so that different datasets (including regulatory inventories and datasets suitable for model development) can be compared. Such comparisons enable regions of overlap and divergence to be identified, as the basis for targeted model development, testing, and/or regulatory action.

It should be noted that the development and application of chemoinformatic methods is an active area of research, and as yet there is no single agreed approach for the use of chemical space analysis in toxicology.

4.2 Analysis of structural features

Structural features were identified either by using SMARTS representations in Knime[8] or subgraph features in the MOSES fingerprinter (Molecular Networks GmbH). The subgraph features, developed by US FDA CFSAN, are grouped by types of atom, bond, ring, functions and connectivity, were coded in the Chemical Substructure Representation Mark-up Language (CSRML) format[9]. CSRML can be used to represent features that cannot be easily written in SMARTS.

Surfactants were classified by a combination of hydrophobic tails and hydrophilic headgroups. Hydrophobic tails include aliphatic chains greater than C8, alkylbenzenes, and polypropylene co-monomer blocks. Hydrophilic head groups include ethyleneoxide chains, carbohydrate, carboxylate, sulfonate, sulphate, phosphate, and quaternary ammonium ions of aliphatic and alkylbenzyl chains.

4.3 Characterisation of Cosmetics Inventory

The application of structural feature analysis to the Cosmetics Inventory is illustrated in Figure 4.1 and Appendix 1.

[8] http://www.knime.org/
[9] http://bulletin.acscinf.org/node/224#W7

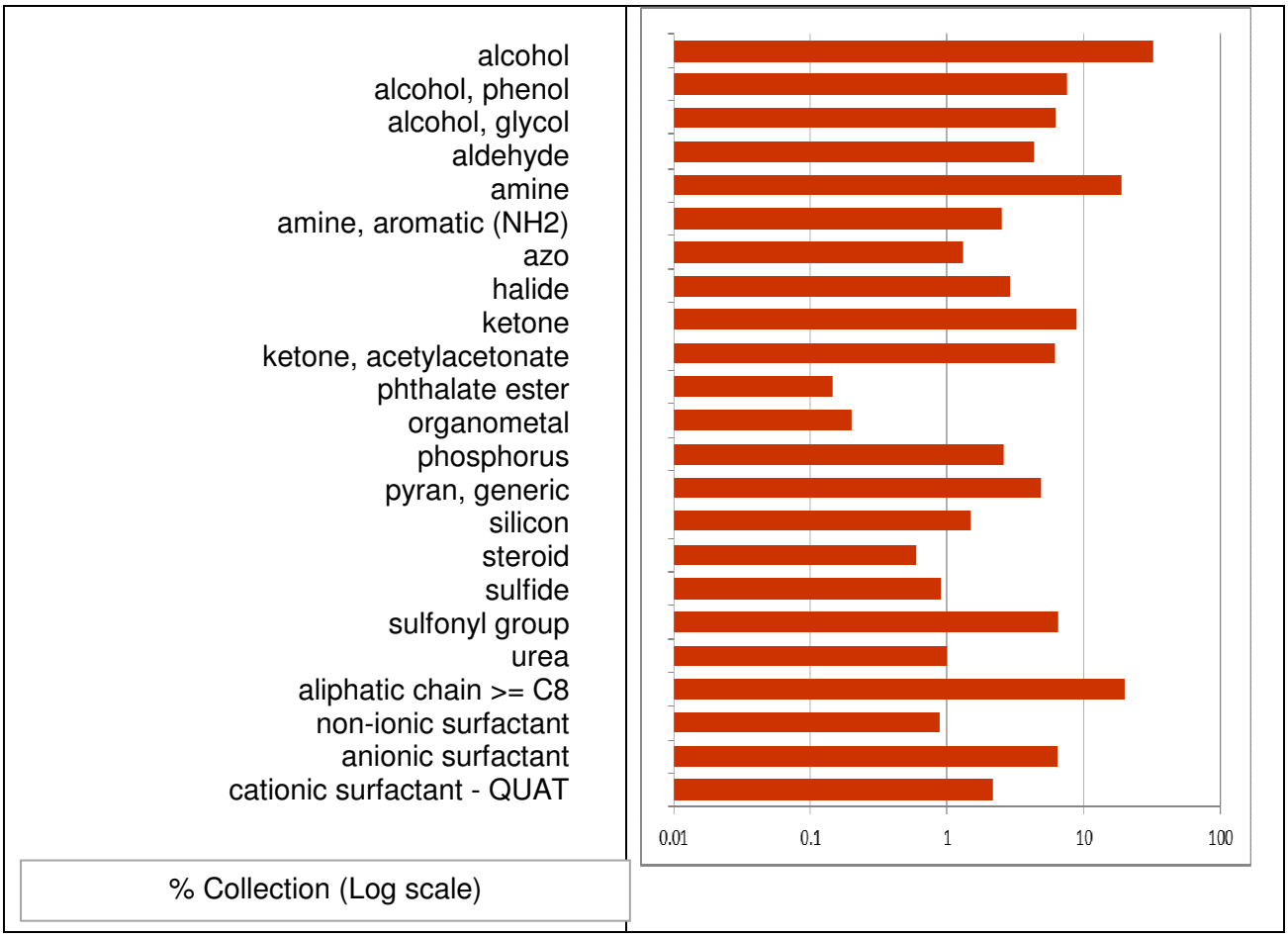

Figure 4.1. Structural domains in the Cosmetics Inventory

4.3 Characterisation of COSMOS TTC dataset

The use of structural feature analysis showed that the COSMOS dataset is lacking in steroids and cationic surfactants (Figure 4.2 and Appendix 1).

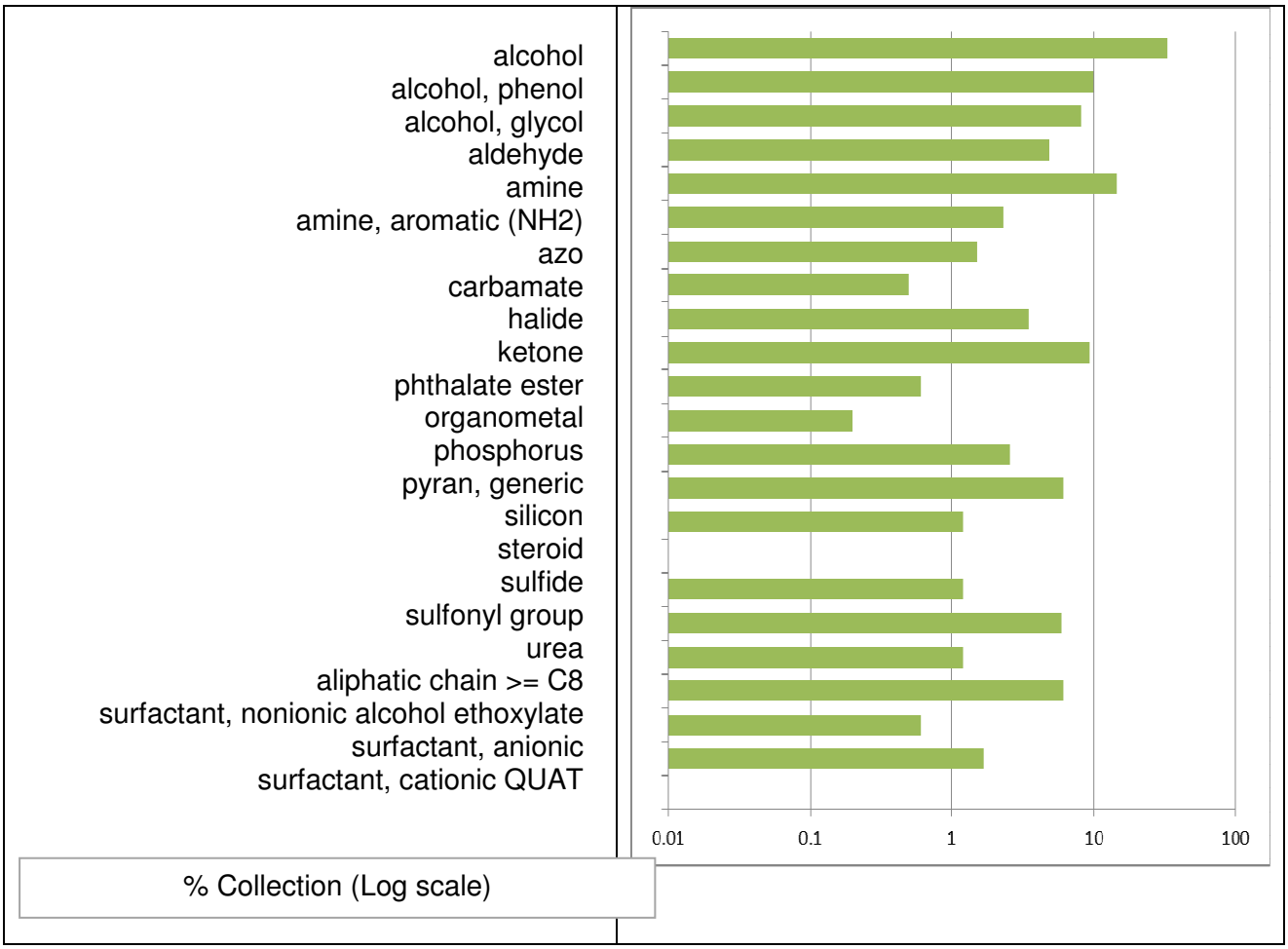

Figure 4.2. Structural domains in the COSMOS TTC dataset

4.4 Characterisation of the Munro TTC dataset

The use of structural feature analysis showed that the Munro dataset is lacking in organometallics, silicon-containing compounds, and notably non-ionic and cationic surfactant classes (Figure 4.3 and Appendix 1). The Munro dataset is also missing acid halides, allenes, boron-containing compounds, and thiocarboxylates (not shown).

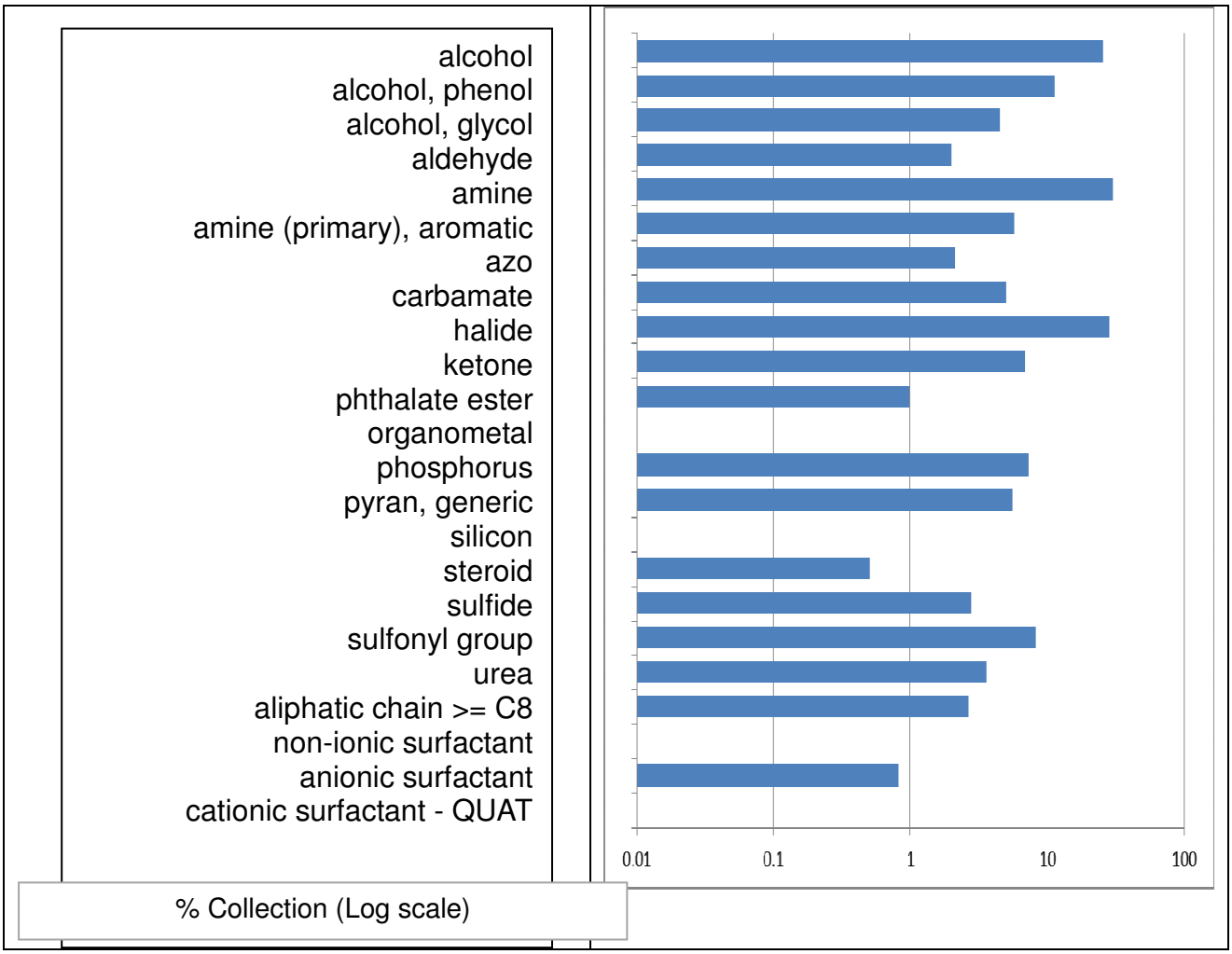

Figure 4.3. Structural domains in the Munro dataset

4.5 Comparison of datasets in terms of structural features

Comparison of the structural analysis across the three datasets (Munro, COSMOS, Cosmetics Inventory) (see above and Appendix 1), showed that the following chemical classes are present in the Cosmetics Inventory but missing in one of the TTC datasets:

a) the COSMOS TTC dataset is missing natural products (steroids) and quaternary ammonium surfactants.

b) the Munro dataset is missing acid halides, allenes, boron-containing compounds, organometals, silicon containing compounds, thiocarboxylates and non-ionic and cationic surfactants.

4.6 Comparison of datasets in terms of physicochemical properties

The three datasets (Munro, COSMOS, Cosmetics Inventory) were characterised in terms of a few key physicochemical properties representing size (molecular weight), shape (diameter, number of rotatable bonds), partitioning behaviour (logP) and reactivity (HOMO and LUMO energies). These physicochemical properties were calculated by using Adriana.Code software (Molecular Networks GmbH, version 2.2.4) and MOPAC (MOPAC2009, Stewart, J.J.P. Stewart Computational Chemistry,

Colorado Springs, CO, USA 2009) for the reactivity descriptors. Statistics for these properties are summarised in Table 4.1.

Table 4.1. Physicochemical property ranges for the Munro and COSMOS datasets and the Cosmetics Inventory

	Munro			COSMOS			Cosmetics Inventory		
	5th percentile	**median**	**95th percentile**	**5th percentile**	**median**	**95th percentile**	**5th percentile**	**median**	**95th percentile**
Molecular weight	85.1	220.7	511.4	92.2	204.3	608.8	60.1	152.1	452.7
LogP	-1.3	2.2	6.2	-1.8	3.0	10.1	-2.6	1.6	5.5
Diameter	4.4	9.6	19.0	5.4	10.9	33.5	3.6	8.6	21.7
No of rotatable bonds	0.0	3.0	11.0	0.0	4.0	28.0	0.0	2.0	14.0
HOMO energy	-11.5	-9.5	-8.3	-11.5	-9.8	-8.4	-11.2	-9.7	-8.5
LUMO energy	-1.9	-0.2	1.6	-1.3	0.4	3.3	-1.2	0.8	3.0

The median values of the molecular weight, the diameter, the number of rotatable bonds and the logP of the Cosmetics Inventory, the Munro and the COSMOS datasets (Table 4.1) were compared by means of a radar chart (Figure 4.3). The analysis of the radar chart and the distribution histograms (Figures 4.4-4.7) showed that:

a) the COSMOS dataset contains smaller (lower molecular weight) structures than the Munro dataset and the Cosmetics Inventory. Despite these differences, more than 85% of the structures have a molecular weight ≤ 400 in the 3 datasets (Figure 4.4).

b) the Cosmetics Inventory has higher prevalence of long linear chain structures (higher number of rotatable bonds and diameter). This finding is confirmed by the distribution of these properties (Figures 4.5 and 4.6)

c) the COSMOS dataset has a higher prevalence of hydrophilic chemicals (lower logP values; Figure 4.7). Calculated logP values greater than 8 or smaller than -8 were not considered as they have no physical meaning.

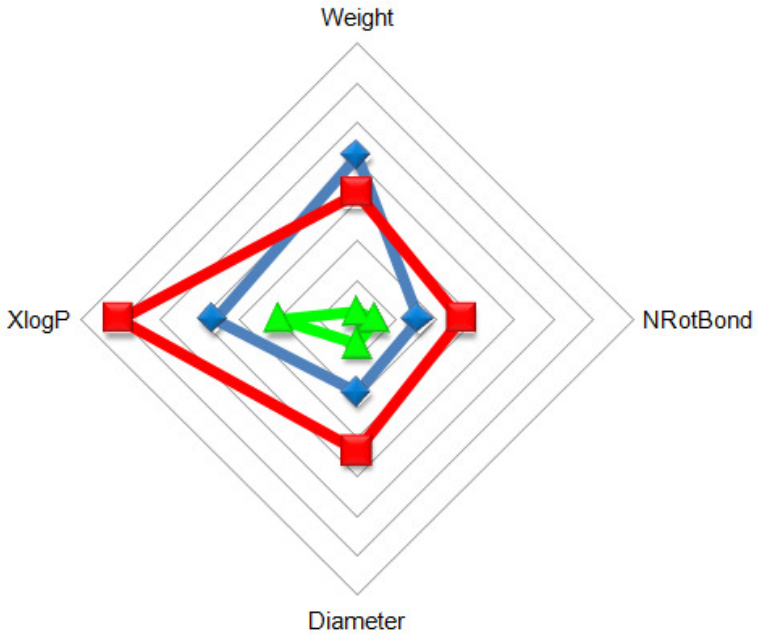

Figure 4.3. Radar chart showing the median values of key physicochemical properties for the Munro and COSMOS datasets and the Cosmetics Inventory. Munro = blue; COSMOS TTC = green; Cosmetics Inventory = red.

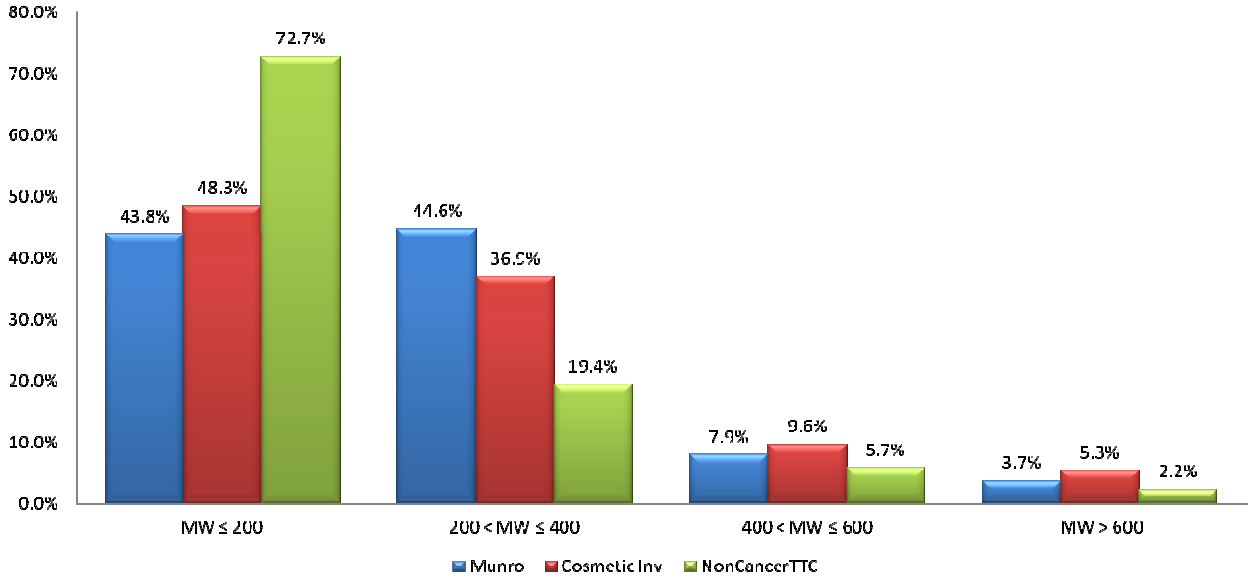

Figure 4.4. Bar chart showing the distribution of molecular weight values across the three datasets. Munro = blue; COSMOS TTC = green; Cosmetics Inventory = red.

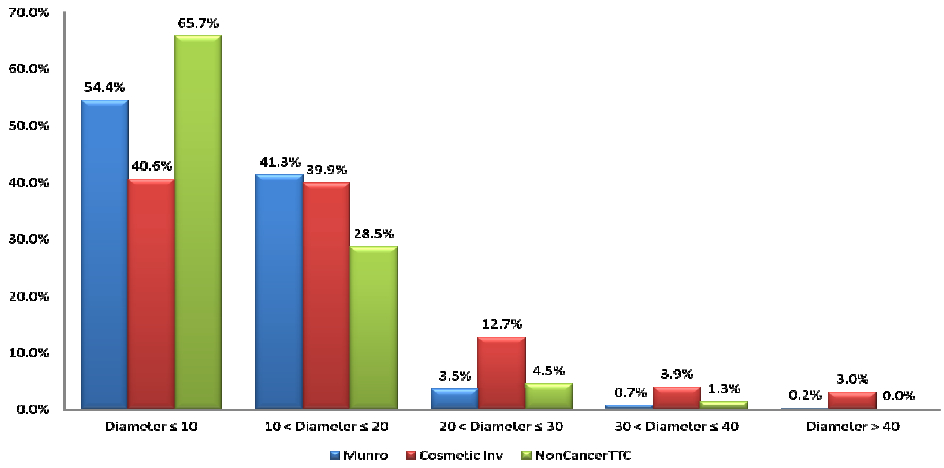

Figure 4.5. Bar chart showing the distribution of molecular diameter values across the three datasets. Munro = blue; COSMOS TTC = green; Cosmetics Inventory = red.

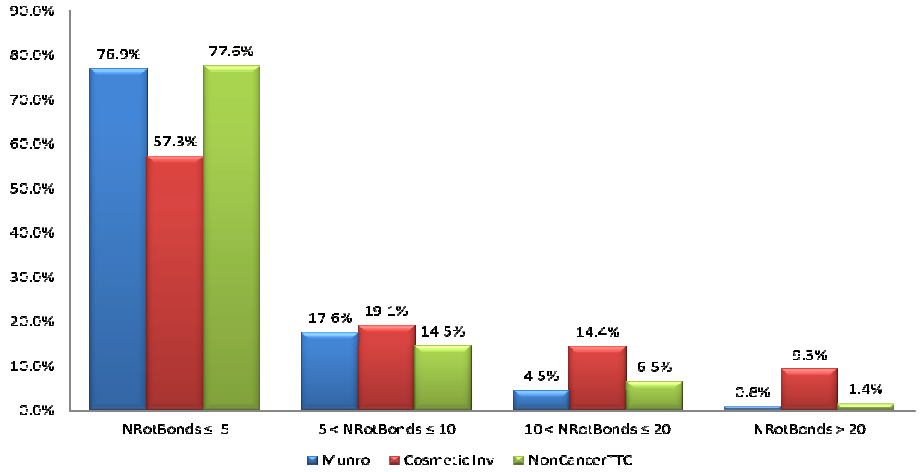

Figure 4.6. Bar chart showing the distribution of rotatable bond values across the three datasets. Munro = blue; COSMOS TTC = green; Cosmetics Inventory = red.

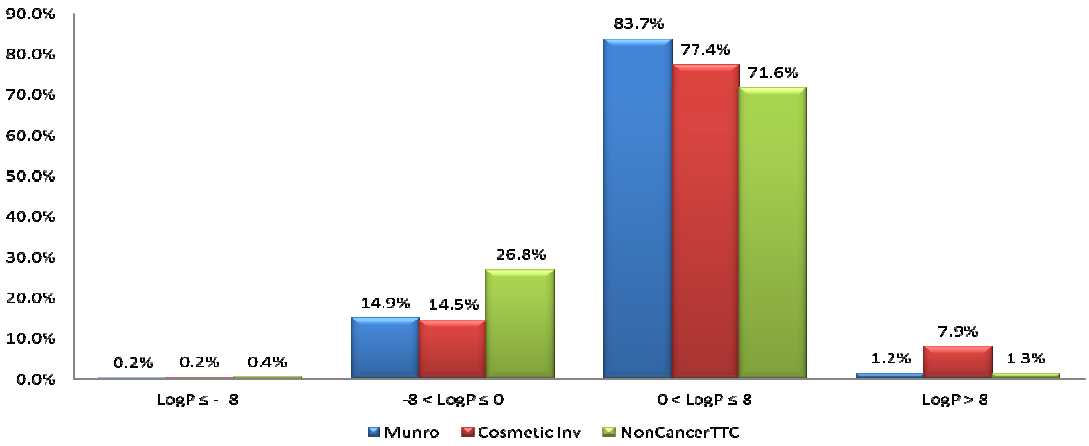

Figure 4.7. Bar chart showing the distribution of logP values across the three datasets. Munro = blue; COSMOS TTC = green; Cosmetics Inventory = red.

Highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) energies were compared by means of the analysis of their distribution (Figures 4.8 and 4.9). HOMO and LUMO are acronyms for and lowest unoccupied molecular orbital, respectively. Both the HOMO and the LUMO energies are important in radical reactions and are to a certain extent related to the reactivity of the molecules.

The HOMO energy is a measure of nucleophilicity: it is directly related to the ionisation potential and characterises the susceptibility of the molecule toward attack by electrophiles. The LUMO energy is a measure of electrophilicity: it is directly related to the electron affinity and characterises the susceptibility of the molecule toward attack by nucleophiles.

Thus, structures with electrons at accessible (near-zero) HOMO levels tend to be good nucleophiles because it does not cost much to donate these electrons when forming a new covalent bond. Similarly, molecules with low LUMO energies tend to be good electrophiles because it does not cost much to place electrons into these orbital.

Figure 4.8 shows that the HOMO distribution is similar across the 3 datasets: there is a comparable percentage of structures in the COSMOS and in the cosmetic inventory with HOMO values greater than -9 (16-18%), whereas this percentage is slightly higher in Munro dataset (25%).

Figure 4.9 shows that the LUMO distributions of the COSMOS and Cosmetics Inventory are similar, while the Munro dataset is characterised by lower LUMO values.

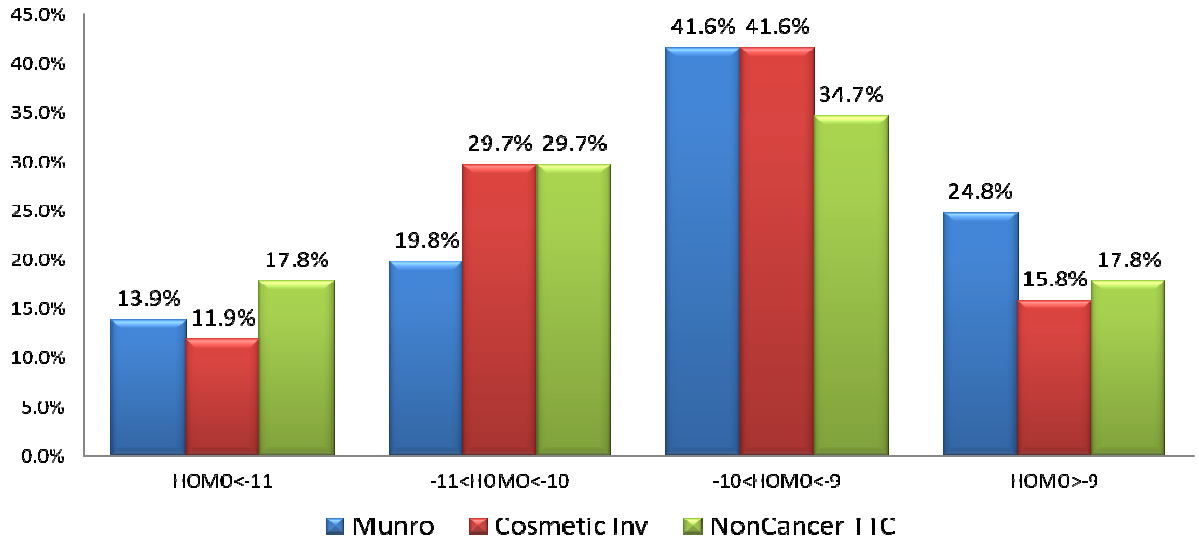

Figure 4.8. Bar chart showing the distribution of HOMO energy values across the three datasets. Munro = blue; COSMOS TTC = green; Cosmetics Inventory = red.

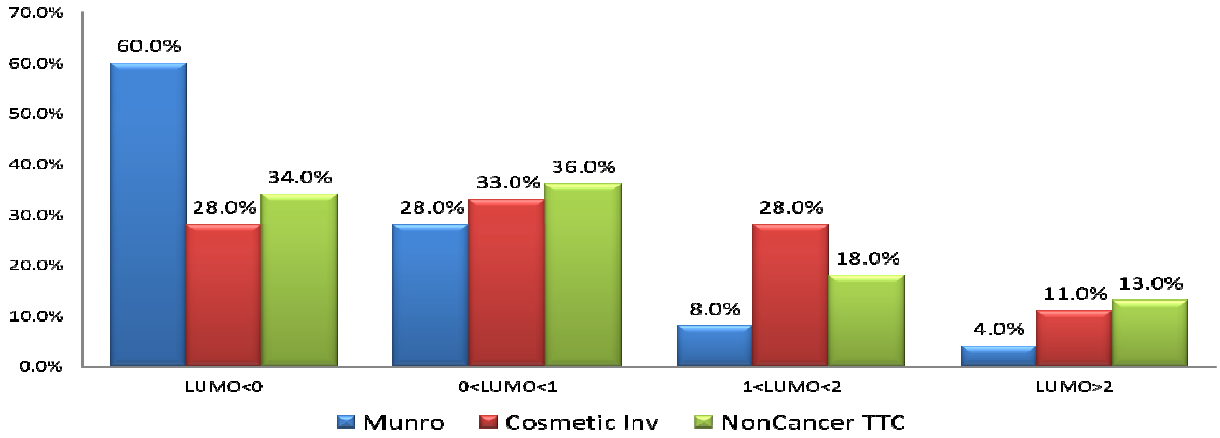

Figure 4.9. Bar chart showing the distribution of LUMO energy values across the three datasets. Munro = blue; COSMOS TTC = green; Cosmetics Inventory = red.

Another way at looking at chemical space is to visualise the overlap between the datasets in a 3D space defined by key descriptors. Figure 4.10 shows that the Munro and COSMOS datasets are overlapping the space defined by complexity, total polar surface area (TPSA) and lipophilicity/partioning (logP). Complexity is a topological descriptor that provides a measure of skeletal complexity as a function of bond connectivities and the diversity of atom types (Hendrickson, 1987). The TPSA of a molecule, defined as the sum of the contributions to the molecular surface area of polar atoms such as oxygen, nitrogen and their attached hydrogens, is a measure of propensity for polar interactions (Prasanna & Doerksen, 2009).

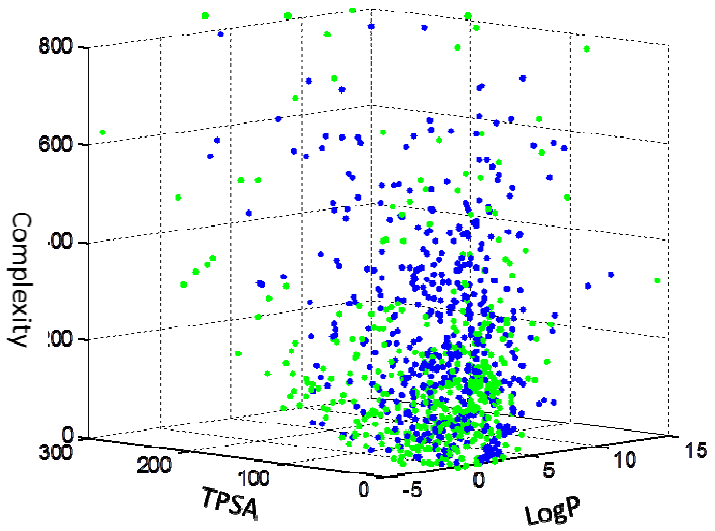

Figure 4.10. 3D plot of physicochemical space between the Munro and COSMOS dataset. Munro = blue; COSMOS = green.

Similarly, Figure 4.11 shows that the Munro and COSMOS datasets are mostly overlapping the space defined by molar volume, solubility (logS) and dipole moment (polarity/reactivity). The COSMOS dataset, tends to have more polar and water soluble structures.

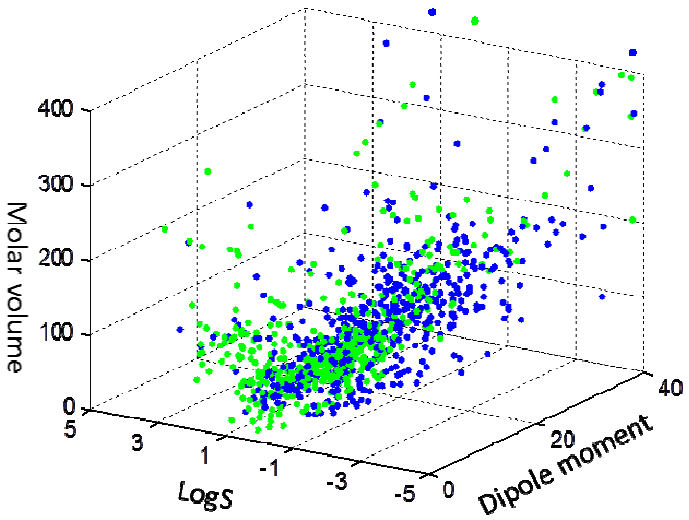

Figure 4.11. 3D plot of physicochemical space between the Munro and COSMOS dataset. Munro = blue; COSMOS = green

The Cosmetics Inventory covers a very diverse range of physicochemical properties. Comparison of the COSMOS TTC dataset with the Cosmetics Inventory in this way shows an overlap between the two datasets (Figures 4.12-4.13), indicating that the TTC dataset is representative of the chemical space of cosmetics in general.

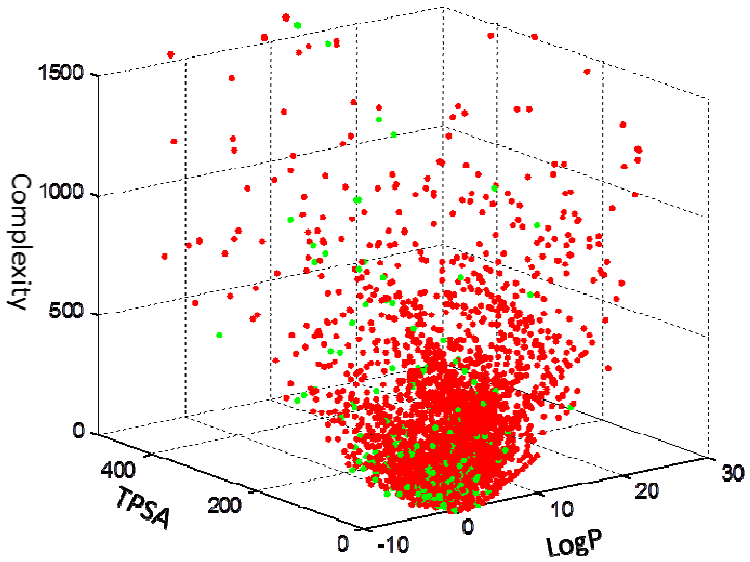

Figure 4.12. 3D plot of physicochemical space between the Cosmetics Inventory and COSMOS TTC dataset. Cosmetics Inventory = red; COSMOS = green.

When plotting the 3-D space of the Cosmetics Inventory defined by logS, dipole moment, and molar volume, several chemical clusters emerged as illustrated in Figure 4.13. The combination of water solubility, polarity/reactivity, and molecular size (volume) seem to separate well-known cosmetics ingredients including the quaternary ammonium alkyl chains, sugar polyols, ethoxylated alcohols, carboxylic esters, alkenes and retinoic acids clusters.

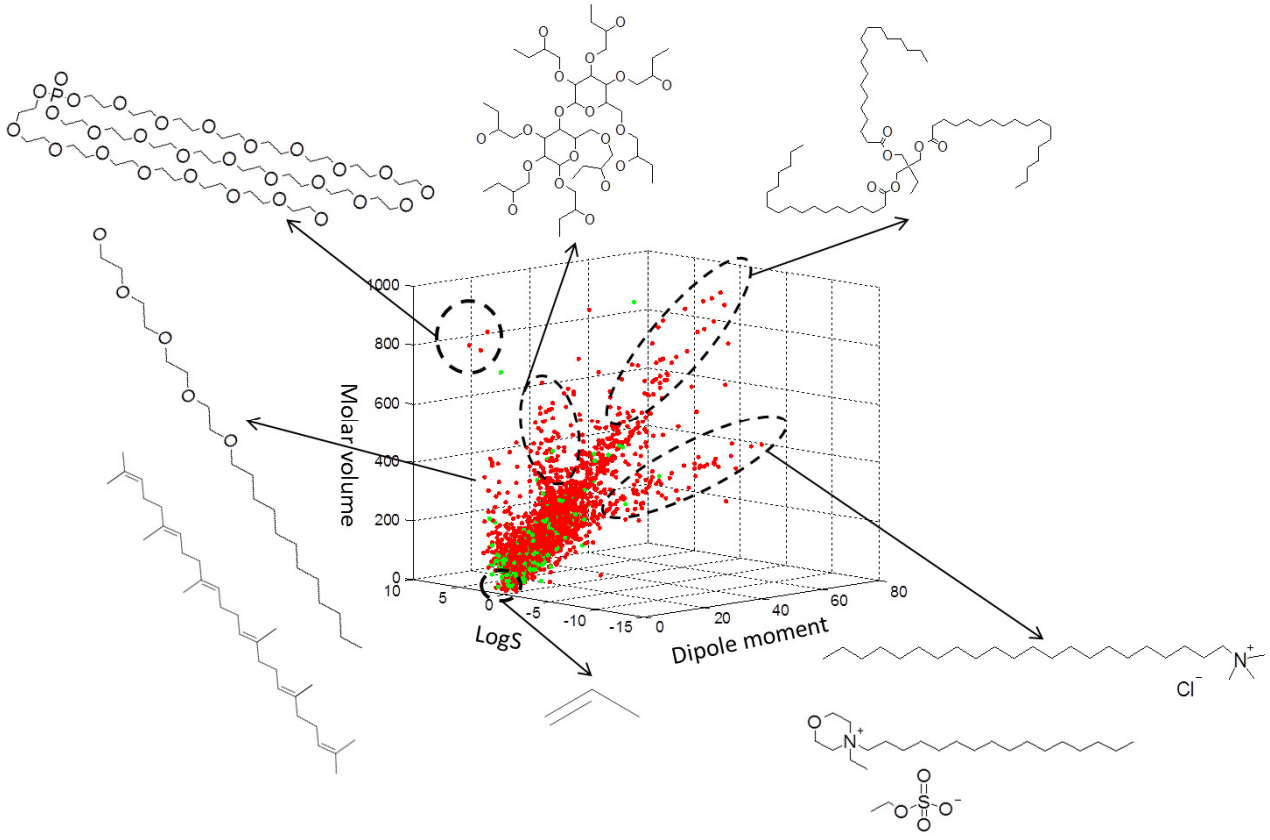

Figure 4.13. 3D plot showing overlap in physicochemical space between the Cosmetics Inventory (red) and COSMOS TTC dataset (blue)

5. Cramer analysis

The chemicals in each dataset were categorised according to their Cramer classification by using the non-extended version of the Cramer tree (v2.5.0). The results for the Munro dataset are illustrated in Figure 5.1. These are compared with the results for the COSMOS dataset and Cosmetics Inventory in Table 5.1. It can be seen that the distribution of chemicals across Cramer classes is similar for the COSMOS dataset and the Cosmetics Inventory, with a fairly even balance between Cramer classes I and III. In contrast, in the Munro dataset, most of the chemicals (75%) are in Cramer class III, with a much lower proportion (21%) being found in Cramer class I. The percentage of chemicals classified in Cramer class II is less than 10%, which is typical of many datasets.

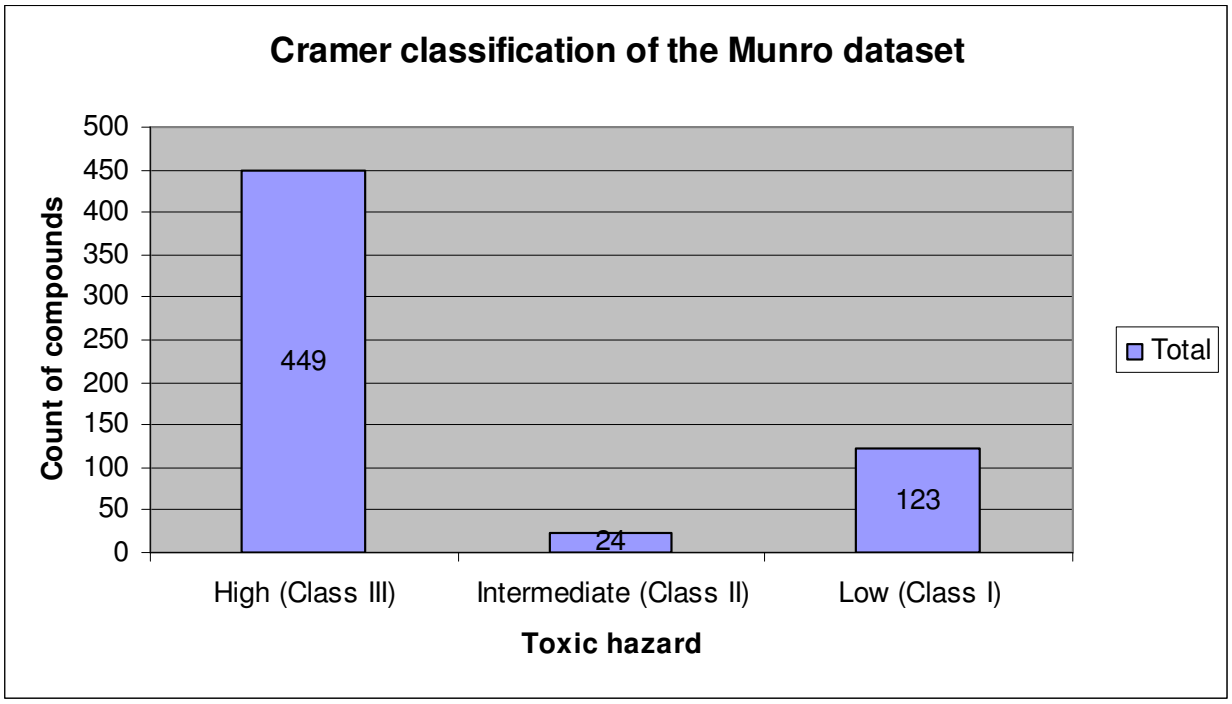

Figure 5.1. Cramer analysis of the Munro data set

Table 5.1. Cramer analysis of the three datasets

Cramer Class	Munro		COSMOS TTC		Cosmetics Inventory	
Class I	123	(21%)	201	(52%)	1977	(45%)
Class II	24	(4%)	34	(9%)	327	(7%)
Class III	449	(75%)	150	(39%)	2154	(48%)
TOTAL	596		385		4458	

6. Toxicity data analysis

6.1 Dataset profiling in terms of DNA-binding, protein-binding and AChE inhibition

The three datasets were analysed in terms of the number and percentage of chemicals in each dataset having structural alerts for DNA-binding or protein-binding. These profilers are based entirely on considerations of mechanistic chemistry (Enoch & Cronin, 2010; Enoch et al, 2011a,b) and can be used to make inferences about potential genotoxicity (in the case of the DNA-binding profiler) and potential skin sensitisation (in the case of the protein-binding profiler). The profilers were coded using the SMARTS representation and implemented in Knime.

The results of this profiling exercise are given in Table 6.1 and Figure 6.1

Table 6.1. Results of dataset profiling with structural alerts for DNA-binding, protein-binding and AChE inhibition

Structural alerts	Munro	COSMOS TTC	Cosmetics Inventory
% DNA-binding	46.1	29.6	27.0
% Protein-binding	28.9	27.8	20.7
% AChE inhibitors	1.7	0.0	0.1
No DNA-binding	275	114	1206
No Protein-binding	172	107	921
No AChE inhibitors	10	0	3
TOTAL	596	385	4460

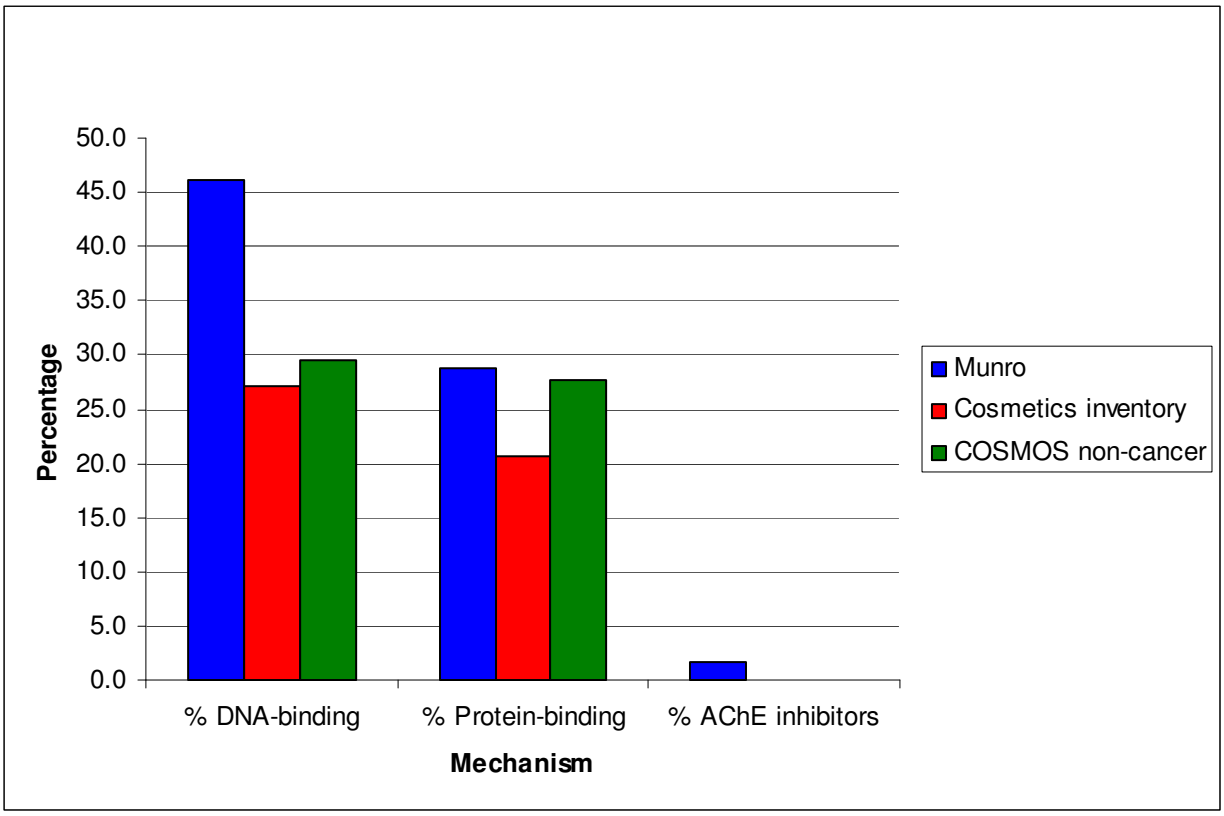

Figure 6.1.Bar charts showing the dataset profiling results based on structural alerts for DNA-binding, protein-binding and AChE inhibition

6.2 Analysis of NOEL distributions in TTC datasets

To assess the degree of protectiveness provided by the Cramer-related (Munro) threshold values for cosmetic ingredients, the Munro threshold values were compared with the corresponding thresholds derived from a cumulative distribution analysis of NOEL values in the COSMOS TTC dataset. As mentioned above, this dataset was derived from mulitple data sources (Munro, PAFA, ToxRefDB and RepDose) and is subject to ongoing extension and revision within COSMOS.

For simplicity, the distribution analysis was applied to the lowest NOEL value for each substance in the dataset, which may not be the NOAEL, i.e. the lowest NOEL for a toxicologically relevant effect. Indeed, the presence of free-standing NOELs may result in an over-conservative estimate of the 5th percentiles.

Distribution analysis was applied to NOEL values for 385 structurally well-defined substances excluding inorganics, organometallics, polymers, and substances for which the tested form was unknown. This analysis included developmental and reproductive toxicity studies, but excluded all repeat dose studies with an exposure duration less than a subchronic study (typically 90 days). The NOEL values from subchronic studies were divided by a factor of 3 (Munro adjustment factor for subchronic to chronic conversion). The 5th percentile NOEL for the substances in each Cramer class was calculated from a theoretical log-normal cumulative distribution. Even though the three cumulative distribution curves (Figure 6.1) were clearly non-normal, this calculation method was considered more robust than a non-parametric approach, since the use of data from the full distribution gives more robust estimates of the percentiles.

The 5th percentiles for the substances in each Cramer class are summarised in Table 6.1. It can be seen that in the case of Cramer Class I, the 5th percentile derived from the COSMOS dataset (1362 µg/kg/day) is lower than the corresponding Munro value (3000 µg/kg/day)[10] by a factor of about 2.

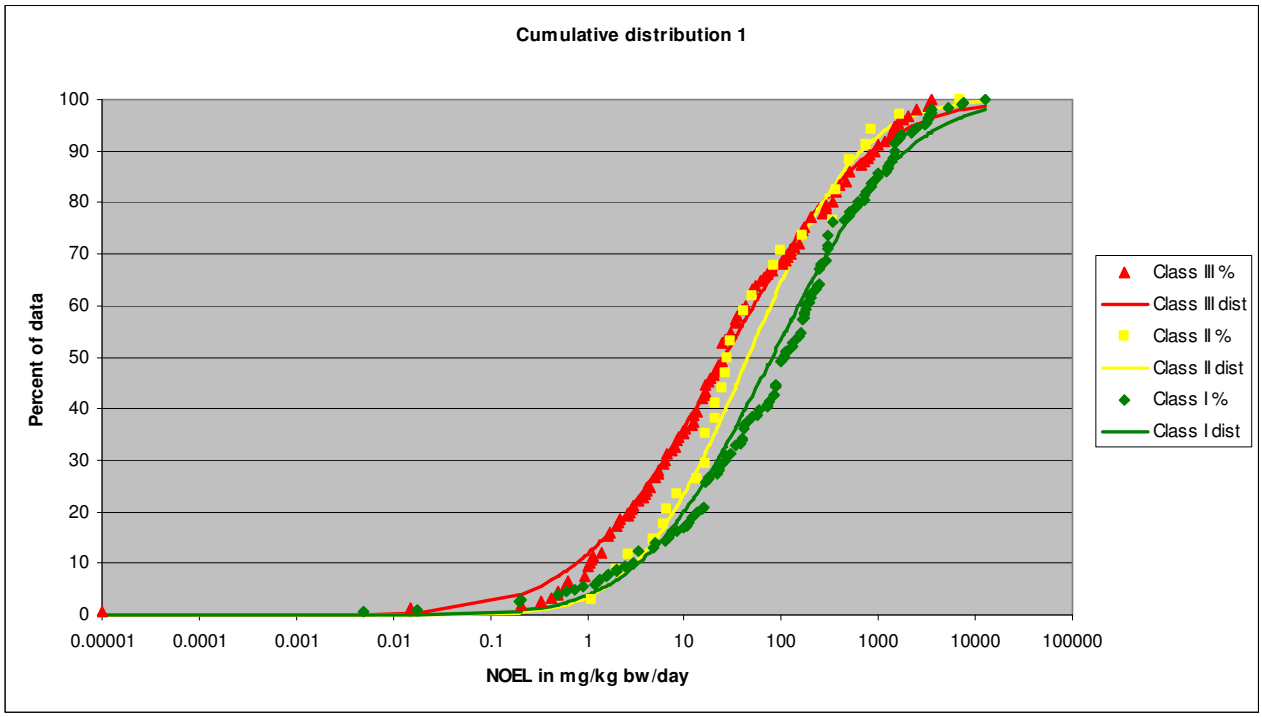

Figure 6.1. Distribution analysis of the COSMOS dataset

Table 6.1. Distribution analysis of COSMOS datasets compared with the Munro thresholds

Cramer class	No. of chemicals	5th percentile NOEL (µg/kg/day)	Human Exposure Threshold (µg/person/day)	Munro 5th percentile NOEL (µg/kg/day)	Munro TTC value (µg/person/day)
Class I	201	**1362**	817	**3000**	1800
Class II	34	**1443**	866	**910**	540
Class III	150	284	170	150	90

A list of Cramer Class I cosmetics for which the lowest NOEL is less than 3000 µg/kg/day is given in Appendix 2; and a list of Cramer Class III cosmetics for which the lowest NOEL is less than the corresponding Munro value of 150 µg/kg/day is given in Appendix 3. These chemicals can be regarded as "false negatives" for Classes I and III, respectively. There were no false negatives for Cramer Class II (i.e. no chemicals for which the lowest NOEL was less than the corresponding Munro value of 910 µg/kg/day). Chemicals having a lowest NOEL greater than their Cramer-related threshold value can be regarded as "true negatives".

The breakdown of true and false negatives for the Cramer classes is shown in Table 6.2. This shows that only two out of 150 Cramer Class III chemicals (i.e. 1.3 %) are false negatives, which is less than

[10] This is the unadjusted value, i.e. calculated before applying the safety factor of 100.

the prevalence of 5% expected by chance. One of these chemicals, biotin, has a lowest NOEL of 0.015 mg/kg/day (15 µg/kg/day), which is a factor of 10 lower than the corresponding Munro value of 150 µg/kg/day. The other chemical, ergocalciferol (vitamin D2), has a lowest NOEL of 0.00001 mg/kg/day (0.01 µg/kg/day), which is a factor of 15,000 lower than the corresponding Munro value of 150 µg/kg/day. Both of these NOELS are free-standing NOELs derived from the PAFA database, and are hence likely to be conservative.

The results also show that 19 out of 201 Cramer Class I (i.e. approx. 9.5%) are false negatives,which is higher than the prevalence of 5% expected by chance. The false negatives are identified in Appendices 2 and 3.

Table 6.2. True and false Cramer class negatives in theCOSMOS TTC dataset

	NOEL threshold		
	Class I (3 mg/kg/day)	**Class II (0.91mg/kg/day)**	**Class III (0.15 mg/kg/day)**
Greater than threshold "True negatives"	182	34	148
Less than threshold "False negatives"	19	0	2
Total	201	34	150

6.3 Removal of substances with structural alerts before Cramer classification

According to the TTC decision tree of Kroes et al (2004), the Cramer scheme should not be applied to genotoxic substances, for which a threshold of 1.5 µg/person/day (0.025 µg/kg/day for a 60kg adult) is proposed. Furthermore, the Cramer scheme should not be applied to chemicals predicted to be neurotoxic as a result of acetylcholinesterase inhibition (organophosphates, carbamates), for which a threshold of 18 µg/person/day (0.3 µg/kg bw/day) is proposed. These proposals are widely accepted (e.g. EFSA, 2011).

It was therefore decided to investigate how many of the Cramer class false negatives had structural alerts for DNA-binding (potential genotoxicity). None of the chemicals in the TTC dataset had structural alerts for acetylcholinesterase inhibition, so this lower threshold was not applied. In addition, since skin sensitisation is of particular concern for cosmetics, the false negatives having structural alerts for protein binding were identified. Several studies have investigated the feasibility of developing TTC values for dermal sensitisation (Safford et al, 2008; Keller et al, 2009). These thresholds are more tentative, and were not applied in this study.

The false negatives for Cramer Classes I and III are identified in Appendices 2 and 3, respectively. There were no false negatives in Cramer Class II.

In terms of their structure, the 19 Cramer Class I false negatives include various alcohols, aliphatic halides, alkenes with conjugated double bonds (e.g. alpha-isomethyl ionone), amines with tertiary and quaternary amino groups (trilaurylamine, carnitine), carboxylic esters (methyl caprylate, butyl acetate), retinoids (retinyl acetate, retinyl palmitate), an aromatic ketone (acetophenone) and an alkyl sulphide (dimethyl sulphide).

As shown in Table 6.3, nine of the 19 Cramer Class I false negatives trigger structural alerts for DNA or protein binding, which means that different thresholds would be applied to these chemicals. Seven of these chemicals, having DNA-binding alerts, have lowest NOEL values in the range 0.2-2.0

mg/kg/day (i.e. 200-2000 μg/kg/day), which is 3-4 orders of magnitude higher than the TTC value for genotoxic chemicals of (0.025 μg/kg/day). In addition, the two retinoids (retinyl acetate, retinyl palmitate) trigger protein-binding alerts. It can be assumed that these chemicals would not be assessed using the Cramer-related thresholds: either they would be assessed by using suitable dermal sensitisation thresholds, or they would be subjected to chemical-specific risk assessment (i.e. TTC would not be applied).

It is also noteworthy that 14 out of the 19 NOEL values for Cramer Class I false negatives are derived from the PAFA database, which is based on a wide range of study types, species and exposure durations, and includes a high percentage (around 45%) of free-standing NOELs. Within the COSMOS project, these data (as well as data from other sources) are being re-evaluated in terms of their toxicological relevance, and it is expected that many of the data points will either be excluded from subsequent versions of the COSMOS TTC dataset, or associated with different (higher) NOEL values.

Another Cramer Class I false negative is isopropyl alcohol, which is derived from the Munro dataset and has a NOEL of 0.018. This result was taken from a 1978 study (Antonova & Salmina, 1978). In contrast to this observation, it has been noted that later developmental toxicity studies using higher doses did not find evidence of teratogenicity (EFSA, 2011). This emphasises the need to critically evaluate at least some of the NOELs in the underlying TTC datasets, in relation to the wider toxicological literature, as well as modern criteria for NOEL selection.

Table 6.3. Structural alerts for false negatives in theCOSMOS TTC dataset

	Class I (3 mg/kg/day)	Class II (0.91mg/kg/day)	Class III (0.15 mg/kg/day)
False negatives	19	0	2
False negatives with DNA binding alerts	7	0	0
False negatives with protein binding alerts	8	0	0
False negatives with DNA AND protein binding alerts	6	0	0
False negatives with DNA OR protein binding alerts	9	0	0

7. Summary, conclusions and recommendations

This report describes the application of chemoinformatic methods to explore the applicability of the Threshold of Toxicological Concern (TTC) approach to cosmetic ingredients. The chemical space of the Munro non-cancer dataset was characterised to assess whether this underlying TTC dataset is representative of the "world" of cosmetic ingredients, as represented by the COSMOS Cosmetics Inventory. In addition, the commonly used Cramer-related Munro threshold values were applied to a toxicological dataset of cosmetic ingredients, the COSMOS TTC dataset, to assess the degree of protectiveness resulting from the application of the Cramer classification scheme. This analysis is considered preliminary, since the COSMOS TTC dataset and Cosmetics Inventory are subject to an ongoing process of extension and quality control within the COSMOS project.

The results of this preliminary analysis show that the Munro dataset is broadly representative of the chemical space of cosmetics, although certain structural classes are missing, notably organometallics, silicon-containing compounds, and certain types of surfactants (non-ionic and cationic classes). Furthermore, compared with the Cosmetics Inventory, the Munro dataset has a higher prevalence of reactive chemicals and a lower prevalence of larger, long linear chain structures. The COSMOS TTC dataset, comprising repeat dose toxicity data for cosmetics ingredients, shows a good representation of the Cosmetics Inventory, both in terms of physicochemical property ranges, structural features and chemical use categories. Thus, this dataset is considered to be suitable for investigating the applicability of the TTC approach to cosmetics.

Analysis of the data in the COSMOS TTC dataset revealed that the 5th percentile in the cumulative probability distribution of NOEL values for Cramer Class I cosmetics is approximately two-fold lower than than the corresponding 5th percentile in the Munro dataset. More specifically, 19 Cramer Class I cosmetics were identified with NOEL values lower than the Munro threshold of 3000 µg/kg bw/day. These were considered as false negatives for Cramer Class I. Within Cramer Class II, there were no false negatives, and within Cramer Class III, there were only two (both vitamins).

While it is a matter for risk managers and regulatory bodies to decide on acceptable levels of protection (including an acceptable level of false negatives), it should be noted that the prevalence of "false negatives" in Cramer Class I was less than 5%, the percentage that would be expected by chance based on the use of the 5th percentile of cumulative probability distribution of NOELs. Furthermore, it was found that the majority of these false negatives do not arise when structural alerts for DNA-binding are used to identify potential genotoxicants, to which a lower TTC value of 0.0025 µg/kg bw/day would typically be applied.

Based on these preliminary results, it is concluded that the current TTC approach, based on the use of Cramer scheme (Cramer et al, 1977) and following the recommendations of Munro et al (1996) and Kroes et al (2004), is broadly applicable to cosmetics. Nevertheless, a number of improvements could be made, through the quality control of the underlying TTC datasets, modest revisions / extensions of the Cramer classification scheme, and the development of explicit guidance on how to apply the TTC approach.

The quality control of the underlying TTC datasets is an important but labour-intensive process, since the repeat-dose toxicity data are typically compiled from a range of different study types, animal species, and exposure durations. Some of the data points can be verified from original sources, whereas others cannot. In addition, many of the NOEL values are study NOELs which may not be the critical NOELs for the leading adverse effect. While an extensive quality control of the toxicity data could not be carried out in this study, this analysis is considered to be conservative, since in many cases, the lowest study NOEL (rather than the NOAEL) was used in the toxicity data analysis.

Ongoing work within the COSMOS project will update the COSMOS TTC dataset in the interests of toxicological consistency and transparency.

The Cramer classification scheme and its applicability in different regulatory areas has been examined in numerous studies (e.g. Bassan et al, 2011; Lapenna & Worth, 2011; Kalkhoff et al, 2011; Pinalli et al, 2011). The results of the current study confirm that there is an opportunity to refine the Cramer classification of various chemical classes, and in particular vitamins (retinoids), aliphatic halides, aromatic amines, aromatic azo compounds, and sulphonates. For example, organohalogen compounds default to Class III simply because the decision tree does not distinguish between these compounds, which may lead to overconservative (false positive) classification. Conversely, sulphonate and sulphamate groups can override the effects of toxic groups such as the azo group, leading to an underconservative (false negative) classification. In principle, the latter could pose a problem in the Cramer assessment of azo hair dyes. However, as with other types of hair dyes (most of which are aromatic amines, including heterocyclic aromatic amines), these tend to be removed from consideration through the prior application of DNA-binding or genotoxicity alerts.

Finally, it is recommended that explicit guidance is developed for the use of defined software tools in the TTC assessment of cosmetics, including explicit inclusion and exclusion criteria, and the use of pre-Cramer filters (e.g. structural alerts or QSARs) and well as the Cramer classification scheme. The TTC approach will be further developed in the COSMOS project.

8. Acknowledgements and Disclaimer

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10. Appendices

Appendix 1. Structural analysis of the three datasets

Structural class	Cosmetics Inventory (4463)		Non-cancer Cosmos TTC (559)		Munro (598)	
	# of hits	%	# of hits	%	# of hits	%
alcohol	1419	31.8	188	33.6	150	25.2
alcohol, phenol	376	8.4	61	10.9	67	11.2
alcohol, glycol	241	5.4	42	7.5	26	4.4
aldehyde	208	4.7	32	5.7	12	2
amine	801	17.9	86	15.4	179	30
amine (primary), aromatic	107	2.4	15	2.7	35	5.9
azo, aromatic	62	1.4	8	1.4	13	2.2
halide, organo	142	3.2	21	3.8	172	28.9
ketone	437	9.8	57	10.2	40	6.7
ketone, acetylactonate	23	0.5	3	0.5	3	0.5
phthalate ester	8	0.2	4	0.7	6	1
organometal	11	0.2	1	0.2	0	0
phosphorus	79	1.8	8	1.4	44	7.4
pyran, generic	232	5.2	37	6.6	33	5.5
silicon	58	1.3	6	1.1	0	0
steroid ring system	35	0.8	0	0	3	0.5
sulfide	41	0.9	7	1.3	17	2.9
sulfonyl group	239	5.4	28	5	48	8.1
urea	38	0.9	8	1.4	22	3.7
aliphatic chain >= C8	910	20.4	33	5.9	15	2.5
surfactant, non-ionic alcohol ethoxylate	68	0.7	5	0.2	0	0
surfactant, anionic	260	8.9	18	2	34	0.8
surfactant, cationic QUAT	91	1.8	1	0	1	0
alcohol, carbohydrate polyol	164	3.7	35	6.3	19	3.2
aminonitrophenol	36	0.8	5	0.9	6	1
aromatic nitro	56	1.3	9	1.6	34	5.7
glycerol triacetate	37	0.8	4	0.7	2	0.3
glycolether, ethylene and propylene	237	5.3	18	3.2	8	1.3
parabens, generic (*o,m,p*)	70	1.6	11	1.97	17	2.9
toluene amines, generic (*o,m,p*)	32	0.7	1	0.2	7	1.2

Highlighted are chemotypes that are specifically relevant to cosmetics. The structural analysis was carried out on the structures for the tested forms, rather than the computational forms.

Appendix 2. Cosmetics in the TTC dataset that are false negatives for Cramer Class I

Chemical name	CAS	Lowest NOEL (mg/kg bw/day)	DNA-binding alert	Protein-binding alert
Acetophenone	98-86-2	0.005	No	No
Isopropyl Alcohol	67-63-0	0.018	No	No
Retinyl Acetate	127-47-9	0.2	No	Yes
P,Alpha-Dimethylstyrene	1195-32-0	0.2	No	No
Glutaral	111-30-8	0.21	Yes	Yes
Triethylene Glycol	112-27-6	0.5	No	No
Butyl Acetate	123-86-4	0.5	No	No
2,6-Xylenol	576-26-1	0.2	Yes	Yes
Dimethyl Sulphide	75-18-3	0.6	No	No
2,4-Hexadienal	142-83-6	0.74	Yes	Yes
Methyl Caprylate	111-11-5	1.2	No	No
Alpha-Isomethyl Ionone	127-51-5	1.37	Yes	Yes
Trilaurylamine	102-87-4	1.67	Yes	No
5-Methyl-Alpha-Ionone	79-69-6	1.97	Yes	Yes
Retinyl Palmitate	79-81-2	2.4	No	Yes
Isoamyl Salicylate	87-20-7	1.57	No	No
Pyridoxine HCl	58-56-0	0.9	No	No
Carnitine	541-15-1	1.3	No	No
Methyl Isoeugenol	93-16-3	2	Yes	Yes

Highlighted are false negatives that would not have been disregarded on the basis of structural alerts

Appendix 3. Cosmetics in the TTC dataset that are false negatives for Cramer Class III

Chemical name	CAS	Lowest NOEL (mg/kg bw/day)	DNA-binding alert	Protein-binding alert
Ergocalciferol (vitamin D_2)	50-14-6 1406-16-2	0.00001	No	No
Biotin (vitamin B_7)	58-85-5	0.015	No	No

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Abstract

This report describes the application of chemoinformatic methods to explore the applicability of the Threshold of Toxicological Concern (TTC) approach to cosmetic ingredients. For non-cancer endpoints, the most widely used TTC approach is the Cramer classification scheme, which categorises chemicals into three classes (I, II and III) depending on their expected level of concern for oral systemic toxicity (low, medium, high, respectively). The chemical space of the Munro non-cancer dataset was characterised to assess whether this underlying TTC dataset is representative of the "world" of cosmetic ingredients, as represented by the COSMOS Cosmetics Inventory. In addition, the commonly used Cramer-related Munro threshold values were applied to a toxicological dataset of cosmetic ingredients, the COSMOS TTC dataset, to assess the degree of protectiveness resulting from the application of the Cramer classification scheme. This analysis is considered preliminary, since the COSMOS TTC dataset and Cosmetics Inventory are subject to an ongoing process of extension and quality control within the COSMOS project.

The results of this preliminary analysis show that the Munro dataset is broadly representative of the chemical space of cosmetics, although certain structural classes are missing, notably organometallics, silicon-containing compounds, and certain types of surfactants (non-ionic and cationic classes). Furthermore, compared with the Cosmetics Inventory, the Munro dataset has a higher prevalence of reactive chemicals and a lower prevalence of larger, long linear chain structures. The COSMOS TTC dataset, comprising repeat dose toxicity data for cosmetics ingredients, shows a good representation of the Cosmetics Inventory, both in terms of physicochemical property ranges, structural features and chemical use categories. Thus, this dataset is considered to be suitable for investigating the applicability of the TTC approach to cosmetics. The results of the toxicity data analysis revealed a number of cosmetic ingredients in Cramer Class I with No Observed Effect Level (NOEL) values lower than the Munro threshold of 3000 µg/kg bw/day. The prevalence of these "false negatives" was less than 5%, which is the percentage expected by chance resulting from the use of the 5^{th} percentile of cumulative probability distribution of NOELs in the derivation of TTC values. Furthermore, the majority of these false negatives do not arise when structural alerts for DNA-binding are used to identify potential genotoxicants, to which a lower TTC value of 0.0025 µg/kg bw/day is typically applied. Based on these preliminary results, it is concluded that the current TTC approach is applicable to cosmetics, although a number of improvements can be made, through the quality control of the underlying TTC datasets, modest revisions / extensions of the Cramer classification scheme, and the development of explicit guidance on how to apply the TTC approach.

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