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(Q)SAR tools for priority setting: A case study with printed paper and board food contact material substances

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ABSTRACT

Over the last years, more stringent safety requirements for an increasing number of chemicals across many regulatory fields (e.g. industrial chemicals, pharmaceuticals, food, cosmetics, …) have triggered the need for an efficient screening strategy to prioritize the substances of highest concern. In this context, alternative methods such as *in silico* (i.e. computational) techniques gain more and more importance. In the current study, a new prioritization strategy for identifying potentially mutagenic substances was developed based on the combination of multiple (quantitative) structure-activity relationship ((Q)SAR) tools. Non-evaluated substances used in printed paper and board food contact materials (FCM) were selected for a case study. By applying our strategy, 106 out of the 1723 substances were assigned 'high priority' as they were predicted mutagenic by 4 different (Q)SAR models. Information provided within the models allowed to identify 53 substances for which Ames mutagenicity prediction already has *in vitro* Ames test results. For further prioritization, additional support could be obtained by applying local i.e. specific models, as demonstrated here for aromatic azo compounds, typically found in printed paper and board FCM. The strategy developed here can easily be applied to other groups of chemicals facing the same need for priority ranking.

1. Introduction

Together with the high and continuously growing number of chemical substances subject to safety assessment, comes the need to establish adequate screening strategies to prioritize those of highest concern for human and/or environmental health. One notable example of a large group of substances urgently requiring a prioritization ranking for in-depth safety evaluation, are those used in food contact materials (FCM). Food contamination due to leakage of substances from FCM has become an increasing source of concern for human health (e.g. Liu et al., 2016; Muncke et al., 2014). Since 2011, an updated list of substances authorized as starting product or additive for the manufacture of plastic FCM is available (European Union, 2011). For non-plastic FCM, however, no harmonized European regulation has been established yet. Although national legislation exists in several Member States for different types of FCM, a broad range of substances currently used in FCM have not been evaluated for their safety (European Parliament, 2016).

Printing inks and paper(board) constitute large groups of non-plastic FCM substances. They are often used in combination and have been at the origin of multiple contamination issues, examples being the isopropylthioxanthone and the 4-methylbenzophenone crises (EFSA, 2005; 2009). Most of the substances that can be present in printed paper and board FCM have not been officially

Abbreviations: AD(I), applicability domain (index); ECHA, European Chemicals Agency; EFSA, European Food Safety Authority; FACET, Flavours, Additives and food Contact materials Exposure Task; FCM, food contact materials; FIG, FACET Industry Group; k-NN, k-Nearest Neighbors; IRFMN, Instituto di Ricerche Farmacologiche Mario Negri; (Q)SAR, (quantitative) structure-activity relationship; RASFF, Rapid Alert System for Food and Feed; SA, structural alert; SMILES, simplified molecular-input line-entry system.

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evaluated for their potential toxicity. Consequently, these non-evaluated substances could give rise to future food crises (Van Bossuyt et al., 2016).

Regarding plastic FCM, the European Food Safety Authority (EFSA) requires a core set of test data in order to be able to evaluate consumer safety of these materials. Genotoxicity data are always requested, regardless of the (estimated) migration level (EFSA, 2012). Indeed, genotoxicity i.e. the ability to cause DNA damage, can induce adverse human health effects including cancer (Claxton et al., 2010). In line with new EFSA Scientific Committee's recommendations on genotoxicity testing strategies, a battery of 2 *in vitro* genotoxicity tests is required, i.e. a gene mutation test in bacteria and an *in vitro* mammalian cell micronucleus test. If one of these tests yields a positive or equivocal result, further (*in vivo*) testing may be needed in order to investigate the genotoxic potential of the substance (EFSA, 2016).

The bacterial reverse mutation assay (Ames test) is the most commonly used *in vitro* test to detect gene mutations (OECD, 1997). Although it is a suitable test to identify gene mutation-inducing chemicals, its technical characteristics (in particular the test duration and the high quantity of test compound required) do not allow testing of >1000 substances in a short period of time at reasonable cost. The same obstacles are also encountered with the other assay required in the genotoxicity testing battery. A promising approach to detect mutagens without animal nor *in vitro* testing lies in the application of *in silico* tools. These computer-assisted methodologies are based on available experimental data, and are increasingly adopted in regulatory toxicology because of their time-, cost- and animal-saving nature. In particular, (quantitative) structure activity relationship ((Q)SAR) systems represent promising predictive computational techniques to evaluate potential genotoxicity and carcinogenicity of chemical substances (Serafimova et al., 2010).

(Q)SARs comprise both statistical QSAR and rule-based SAR systems. Rule-based models perform predictions via detection of so-called 'structural alerts' (SA), i.e. chemical fragments responsible for the toxic effect as determined earlier based on human expert knowledge. Statistical models, on the other hand, predict toxicity using an algorithm obtained by investigating the mathematical correlation between chemical properties (translated into molecular descriptors) and toxic activity (Bakhtyari et al., 2013). In both systems, chemicals are typically processed by means of their simplified molecular-input line-entry system (SMILES) representation. Most commercial (e.g. Derek Nexus®) and free (e.g. Toxtree) *in silico* software programs include statistical QSAR and/or rule-based SAR models to predict the induction of gene mutations in the Ames test ('Ames mutagenicity'). Furthermore, due to the abundance of consistent Ames test results and due to the binary result type: mutagenic/non-mutagenic, robust models for Ames mutagenicity are available and therefore the prediction performance for this endpoint is substantially better compared to other toxicological endpoints (Kamath et al., 2015). Indeed, *in silico* models for genotoxic endpoints other than Ames mutagenicity (e.g. chromosome-damaging potential in the micronucleus test) exist, but until now their accuracy is limited and needs to be improved before these models can become a more reliable screening tool.

Numerous publications on (Q)SAR evaluation of chemicals/chemical groups are available, however mostly in the context of model validation. Besides one study in which 2 SAR models were used to rank heat-generated food contaminants (Cotterill et al., 2008), to our knowledge, no study reports are available on the application of (Q)SARs for prioritization of potential human genotoxicants. In the current study, a screening strategy based on (Q)SAR tools is applied to identify, within the large number of non-evaluated substances that can be used in printed paper and board FCM, those that represent the highest concern for human health. The non-evaluated substances were first selected from a recently compiled inventory containing all substances which may be used in this type of FCM (Van Bossuyt et al., 2016). Next, their potential to induce gene mutations was predicted using a battery of Ames mutagenicity (Q)SAR models. The models were selected by taking into account existing recommendations such as the use of complementary systems (in terms of prediction method). Moreover, the combination of a SAR and a QSAR is already mandatory in certain regulatory domains, for example in the case of impurity testing of pharmaceuticals as described in the ICH M7 guidelines (ICH, 2014). Using the combined (Q)SAR results, a priority list could be composed of non-evaluated printed paper and board FCM substances requiring an urgent in-depth safety evaluation.

2. Materials and methods

2.1. Study substances

Substances that have not been officially evaluated were selected from a recently compiled inventory including 6073 unique substances which may be used in printed paper and board FCM (Van Bossuyt et al., 2016). Out of the 4690 non-evaluated compounds, 1769 single substances were retained for the current analysis. The remaining 2921 non-evaluated substances are not eligible for straightforward *in silico* processing, due to their chemical structure (e.g. polymers, mixtures, complexes, inorganic substances). Subsequently, the ChemSpider (Royal Society of Chemistry, 2016), ChemIDplus (National Institutes of Health (2016a)), PubChem (National Institutes of Health (2016b)) and European Chemicals Agency (ECHA, 2016) databases were consulted to collect missing CAS numbers and SMILES for the 1769 non-evaluated single substances. ChemSpider was used as the primary information source, whereas the ChemIDplus, PubChem and ECHA databases were consulted in case ChemSpider yielded no or ambiguous results. Afterwards, the compound selection was further refined by excluding substances for which no definite CAS number or SMILES could be identified, reducing the final number to 1723 (Fig. 1).

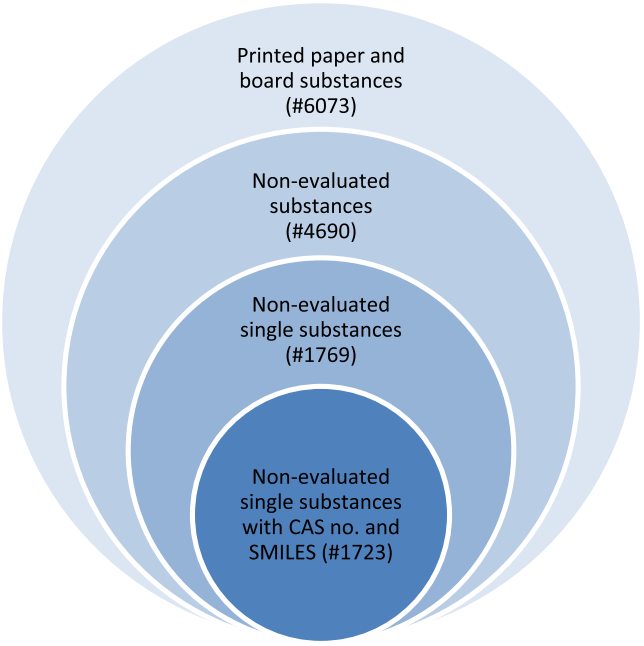

Fig. 1. Selection of study substances.