**Safeguarding human health using *in silico* tools?**

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During the last years, the 3Rs (Replacement, Reduction, Refinement) principle is increasingly taken into consideration in setting up integrated testing strategies. As such, *in silico* methods received substantial attention, which stimulated their development and made them become more interesting for the assessment of chemical hazards. *In silico* tools are essentially computer models, able to make predictions for a non-evaluated compound based on knowledge extracted from a collection of structurally-related substances with experimental toxicity data. Progressively acknowledged by regulatory bodies, *in silico* tools are gaining importance in toxicology not only as a first tier screening tool, but also for complementing *in vivo* and *in vitro* test results (for example Buist et al. 2013, Nendza et al. 2013, Schilter et al. 2014, Scholz et al. 2013). Their widespread use, however, remains limited due to (i) the non-flexibility of the current regulatory framework, strictly describing the required experimental tests, (ii) the oversupply of computer models while often uncertainty exists as to which model (combination) is most suitable to assess a given (type of) substance for a particular endpoint, and (iii) the rather poor predictive capacity for toxicological endpoints other than Ames mutagenicity (Barber and Myatt 2016).

Therefore, the most promising application of *in silico* tools today remains its use in priority setting upon screening of a large number of compounds. The general public is exposed, intentionally or not, to a large variety of different substances, sometimes not or not recently evaluated for their safety. Environmental pollutants or food contaminants are evident examples of non-intentional exposure to predominantly non-evaluated substances. A detailed characterization of the complete toxicological profile of all these substances is not feasible from an economic and ethical (animal welfare) point of view. *In silico* tools, however, can provide substantial help in assigning priority to those substances for which a comprehensive safety evaluation is most urgently needed.

In a recent study, we illustrated the potential of *in silico* tools for such priority setting in the field of paper and board food contact materials (FCM). Paper and board FCM, like other non-plastic FCMs, can contain a large number of non-evaluated substances (European Parliament 2016, Liu et al. 2016, Muncke et al. 2014, Van Bossuyt et al. 2016). Several food crises have confirmed that FCM substances can migrate into food and drinks, subsequently causing unwanted exposure of the consumer to potentially harmful substances (European Commission 2016, EFSA 2011). As a result, health concerns have been raised which are justified especially since migration from FCM is estimated to be the main source of food contamination, quantitatively exceeding most others - including pesticide residues - by a factor of 100-1000 (Grob et al. 2006).

The study focused on Ames mutagenicity, an important toxicological endpoint related to serious adverse health effects including carcinogenicity. Furthermore, for Ames mutagenicity, a number of valid *in silico* models are available to make substantiated predictions. Four different available *in silico* tools were used to select currently non-evaluated printed paper and board FCM substances that most likely exhibit mutagenic properties (Van Bossuyt et al., under review). In particular the substances identified as being of ‘highest priority’ need immediate further investigation. By identifying substances of highest concern, the resources available for experimental testing can be attributed in a more efficient way. Indeed, it would be impossible to carry out elaborate toxicological investigations for hundreds of chemicals in the course of an acceptable time span.

Similarly, *in silico* tools can be of particular interest to screen ‘non-intentionally added substances’ (NIAS) migrating from FCM. These NIAS include impurities, oligomers and degradation products (Muncke 2011, Nerin et al. 2016). NIAS typically represent the larger part of the migrants and their exact composition is often unknown (Grob 2014). In this context, we recently reported on the use of *in silico* tools as part of a strategy to identify non-authorized chemicals of genotoxic concern found to migrate from plastic baby bottles used as alternative to bisphenol A-containing polycarbonate baby bottles (Mertens et al. 2016). Also in this case study, the importance of *in silico* tools for prioritization of FCM substances was clearly demonstrated.

This prioritization strategy based on *in silico* methodology can also be applied in several other domains where there is a need to identify priority substances requiring (geno)toxicological evaluation. Actual examples include compounds used in tattoo inks, permanent make-up, printed baby napkins and sanitary towels, medical devices, textile products and nanomaterials. In all these cases, human health safeguarding can be realized already to some extent without the use of experimental (animal) systems.

**References**

Barber, C.G. and Myatt, G.J. (ed.), 2016. *17th International Conference on QSAR in Environmental*

*and Health Sciences (QSAR 2016),* conference proceedings, 13-17 June, Miami Beach, USA, SAR QSAR Environ. Res. 27, 781-948 [Special edition]

Buist, H., Aldenberg, T., Batke, M., Escher, S., Klein Entink, R., Kühne, R., Marquart, H., Pauné, H.,

Rorije, E., Schüürmann, G., Kroese, D., 2013. The OSIRIS Weight of Evidence approach: ITS mutagenicity and ITS carcinogenicity. Regul. Tox. Pharm. 67, 170-181. <http://dx.doi.org/10.1016/j.yrtph.2013.01.002>

EFSA, 2011. Report of ESCO WG on Non-plastic Food Contact Materials. Available from:

http://www.efsa.europa.eu/sites/default/files/scientific\_output/files/

main\_documents/139e.pdf

European Commission, 2016. Rapid Alert System in Food and Feed. Available from:

<http://ec.europa.eu/food/safety/rasff/portal/index_en.htm>

European Parliament, 2016. Food Contact Materials - How to Ensure Food Safety and Technological

Innovation in the Future? Available from: [http://www.europarl.europa.eu/RegData/etudes/STUD/2016/578967/IPOL\_STU(2016)578967\_EN.pdf](http://www.europarl.europa.eu/RegData/etudes/STUD/2016/578967/IPOL_STU%282016%29578967_EN.pdf)

Grob, K., Biedermann, M., Scherbaum, E., Roth, M., Rieger, K., 2006. Food contamination with

organic materials in perspective: packaging materials as the largest and least controlled source? A view focusing on the European situation. Cri. Rev. Food Sci. Nutr. 46, 529-535. <http://dx.doi.org/10.1080/10408390500295490>

Grob, K., 2014. Work plans to get out of the deadlock for the safety assurance of migration from

 food contact materials? A proposal. Food Control 46, 312-318. <http://dx.doi.org/10.1016/j.foodcont.2014.05.044>

Liu, R., Lin, Y., Hu, F., Liu, R., Ruan, T., Jiang, G., 2016. Observation of emerging photoinitiator

additives in household environment and sewage sludge in China. Environ. Sci. Technol. 50,

97-104. <http://dx.doi.org/10.1021/acs.est.5b04977>

Mertens, B., Simon, C., Van Bossuyt, M., Onghena, M., Vandermarken, T., Van Langenhove, K.,

Demaegdt, H., Van Hoeck, E., Van Loco, J., Vandermeiren, K., Covaci, A., Scippo, M-L., Elskens, M., Verschaeve, L., 2016. Investigation of the genotoxicity of substances migrating from polycarbonate replacement baby bottles to identify chemicals of high concern. Food Chem. Toxicol. 89, 126-137. <http://dx.doi.org/10.1016/j.fct.2016.01.009>

Muncke, J., 2011. Endocrine disrupting chemicals and other substances of concern in food contact materials: An updated review of exposure, effect and risk assessment. J. Steroid Biochem. Mol. Biol. 127, 118-127. <http://dx.doi.org/10.1016/j.jsbmb.2010.10.004>

Muncke, J., Myers, J.P., Scheringer, M., Porta, M., 2014. Food packaging and migration of food

contact materials: will epidemiologists rise to the neotoxic challenge? J. Epidemiol. Community Health. 0, 1–3. <http://dx.doi.org/10.1136/jech-2013-202593>

Nendza, M., Gabbert, S., Kühne, R., Lombardo, A., Roncaglioni, A., Benfenati, E., Benigni, R., Bossa,

C., Strempel, S., Scheringer, M., Fernández, A., Rallo, R., Giralt, F., Dimitrov, S., Mekenyan, O., Bringezu, F., Schüürmann, G., 2013. A comparative survey of chemistry-driven in silico methods to identify hazardous substances under REACH. Regul. Tox. Pharm. 66, 301-314. <http://dx.doi.org/10.1016/j.yrtph.2013.05.007>

Schilter, B., Benigni, R., Boobis, A., Chiodini, A., Cockburn, A., Cronin, M.T.D., Lo Piparo, E., Modi, S.,

Thiel, A., Worth, A., 2014. Establishing the level of safety concern for chemicals in food without the need for toxicity testing. Regul. Tox. Pharm., 68, 275-296. <http://dx.doi.org/10.1016/j.yrtph.2013.08.018>

Scholz, S., Sela, E., Blaha, L., Braunbeck, T., Galay-Burgos, M., García-Franco, M., Guinea, J., Klüver,

N., Schirmer, K., Tanneberger, K., Tobor-Kapłon, M., Witters, H., Belanger, S., Benfenati, E., Creton, S., Cronin, M.T., Eggen, R.I., Embry, M., Ekman, D., Gourmelon, A., Halder, M., Hardy, B., Hartung, T., Hubesch, B., Jungmann, D., Lampi, M.A., Lee, L., Léonard, M., Küster, E., Lillicrap, A., Luckenbach, T., Murk, A.J., Navas, J.M., Peijnenburg, W., Repetto, G., Salinas, E., Schüürmann, G., Spielmann, H., Tollefsen, K.E., Walter-Rohde, S., Whale, G., Wheeler, J.R., Winter, M.J., 2013. A European perspective on alternatives to animal testing for environmental hazard identification and risk assessment. Regul. Tox. Pharm. 67, 506-530. <http://dx.doi.org/10.1016/j.yrtph.2013.10.003>.

Van Bossuyt, M., Van Hoeck, E., Vanhaecke, T., Rogiers, V., Mertens, B., 2016. Printed paper and

board food contact materials as a potential source of food contamination. Regul. Tox. Pharm. 81, 10-19. <http://dx.doi.org/10.1016/j.yrtph.2016.06.025>.

Van Bossuyt, M., Van Hoeck, E., Raitano, G., Manganelli, S., Braeken, E., Ates, G., Vanhaecke, T.,

Van Miert, S., Benfenati, E., Mertens, B., Rogiers, V. (Q)SAR tools for priority setting: a case

study with printed paper and board food contact material substances. *Under review*