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Comparison of assessments of overall toxicity of chemical reactions upon using cytotoxicity and acute toxicity data

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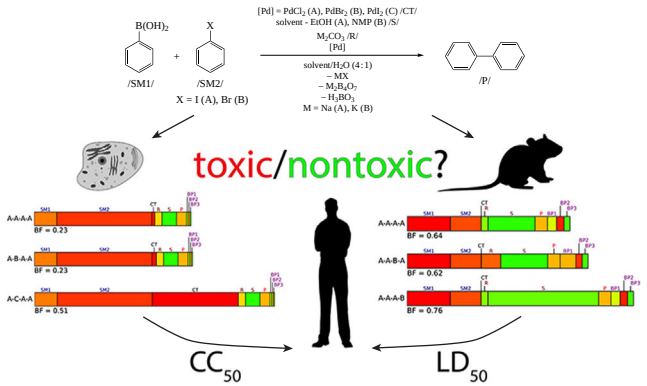
In this work, by means of bio-Strips and toxicity potentials, we compare the results of analysis of toxic potentials of chemical reactions when using cytotoxicity or acute toxicity data. Here, 24 routes of synthesis of 1,1′-biphenyl are used for exemplary purposes.



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In our previous works, we have developed and discussed the benefits of using the cytotoxicity of chemical substances as the basis for preliminary assessment of the potential toxic effects of various chemical processes on the environment and humans.[1,2] Still, the issue of extrapolating the obtained results on higher organisms has not been solved. Among mammals, rats are considered the top model organisms for studying the toxicity of various chemical substances – from the viewpoints of their relative physiological and metabolic similarity to humans and of the relative easiness of their breeding and management in laboratories.[3,4] Unsurprisingly, the globally accepted toxicity ratings (including the Globally Harmonized System of Classification and Labelling of Chemicals (GSH)[5] issued by the United Nations, the WHO Recommended Classification of Pesticides by Hazards[6] issued by the World Health Organization, and the Acute Toxicity Categories[7] issued by the Office of the Federal Register of the United States government) rely on the data on various types of acute toxicity of chemicals studied in rats. Thus, median lethal doses (LD_{50}) of substances measured upon different administration routes (oral, intraperitoneal, intravenous, *via* contact with skin or inhalation) in these rodents are often regarded the most reliable approximations of the harmful doses of the corresponding substances for humans.[8]

Upon looking at the data on acute toxicity of chemicals in mammals available in the NCBI PubChem database[9] or safety data sheets of major chemical suppliers, it can be readily seen that even the most widespread compounds often lack any information on their possible toxic effects. Since experiments in animals are more expensive and complicated than tests *in vitro* and are also associated with ethical concerns, we have suggested using half-maximal cytotoxicity concentrations (CC_{50}) of chemicals for estimating the contribution of participant substances into 'overall toxicity' of chemical reactions processes by means of bio-Profiles (bio-Strips) and the accompanying metrics (bio-Factors and cytotoxicity potentials).[1,2] The evidences of the correlation between the cytotoxicity of various chemical compounds and their lethal plasma concentrations in humans have been found.[10] Still, the comparison of the results of such estimations carried out using cytotoxicity and acute toxicity in animals is demanded. Here, we fill this gap by example of the model reaction of synthesis of 1,1′-biphenyl from phenylboronic acid and aryl halides. In total, 24 synthetic routes are analyzed using bio-Strips and (cyto)toxicity potentials calculated on the basis of toxicity data for the reaction compounds measured in three cell lines and rats upon oral administration.

Experimental 24-h CC_{50} (half-maximal cytotoxicity concentration measured upon 24-h incubation) and LD_{50} (measured in rats upon oral administration) values used in this study were established previously or taken from the available literary sources[2,9] (see Table S1 in Online Supplementary Materials). On the basis of these data, bio-Strips of the chemical reactions and their bio-Factors (BFs) and initial, final, and relative final toxicity potentials (TP_i, TP_f, and TP_{f_rel}, respectively) were calculated (see Table S2). The detailed procedure is provided in Online Supplementary Materials. Here, we used LD_{50} values expressed as mmol per kilogram of body weight (mmol kg^{-1} b.w.) to match the corresponding 24-h CC_{50} values expressed as mmol per liter (mM).

In the routes of synthesis of 1,1′-biphenyl under consideration, the following reaction components are varied: (i) starting material 2 [SM2: iodobenzene (A) or bromobenzene (B)], (ii) catalyst [CT: $Pd(OAc)_2$ (A), $PdCl_2$ (B), or $Pd(acac)_2$ (C)],

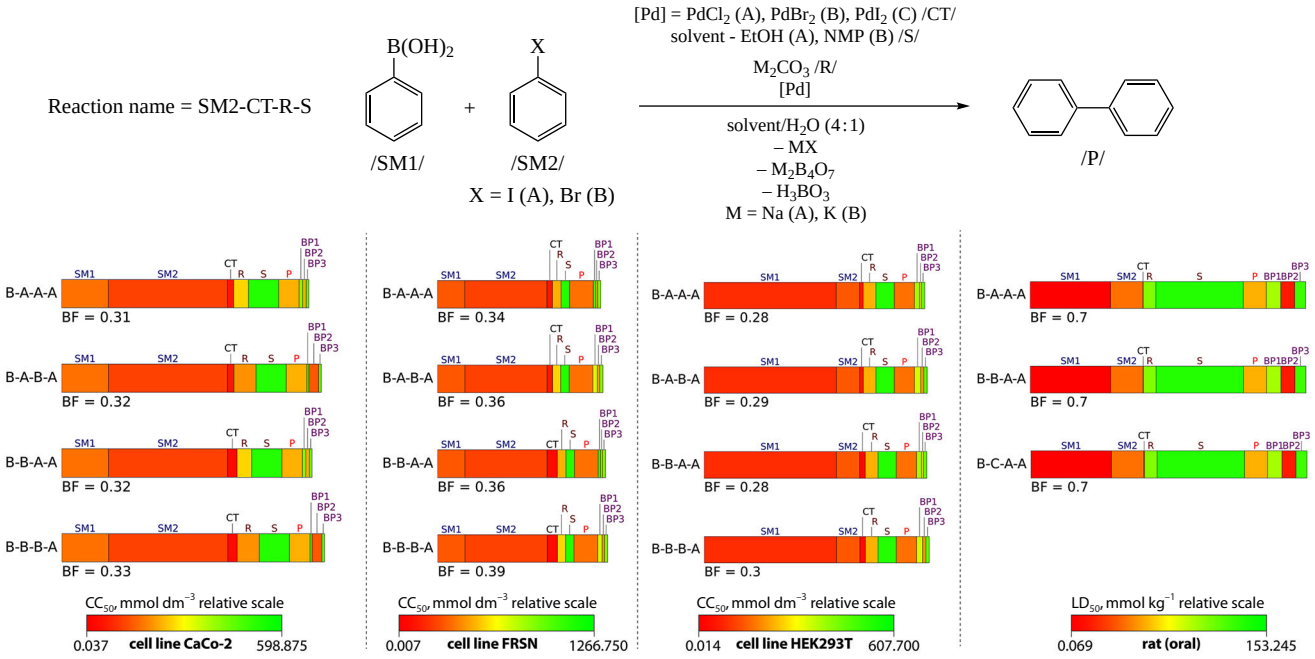

Figure 1 Bio-Strips of the most promising routes of synthesis of 1,1′-biphenyl, according to the cytotoxicity of the corresponding compounds in CaCo-2, FRSN, and HEK293T cell lines, and to acute toxicity upon oral administration in rats. The 1st, 2nd, 3rd, and 4th letters in the reaction names correspond to the types of starting material 2 (SM2), catalyst (CT), reagent (R), and solvent (S), respectively. The color of the bio-Strip sections reflects the CC_{50} of a particular substance measured in a particular cell line or LD_{50} measured in rats (see toxicity scales below the bio-Strips). bio-Factors (BFs) are also shown.

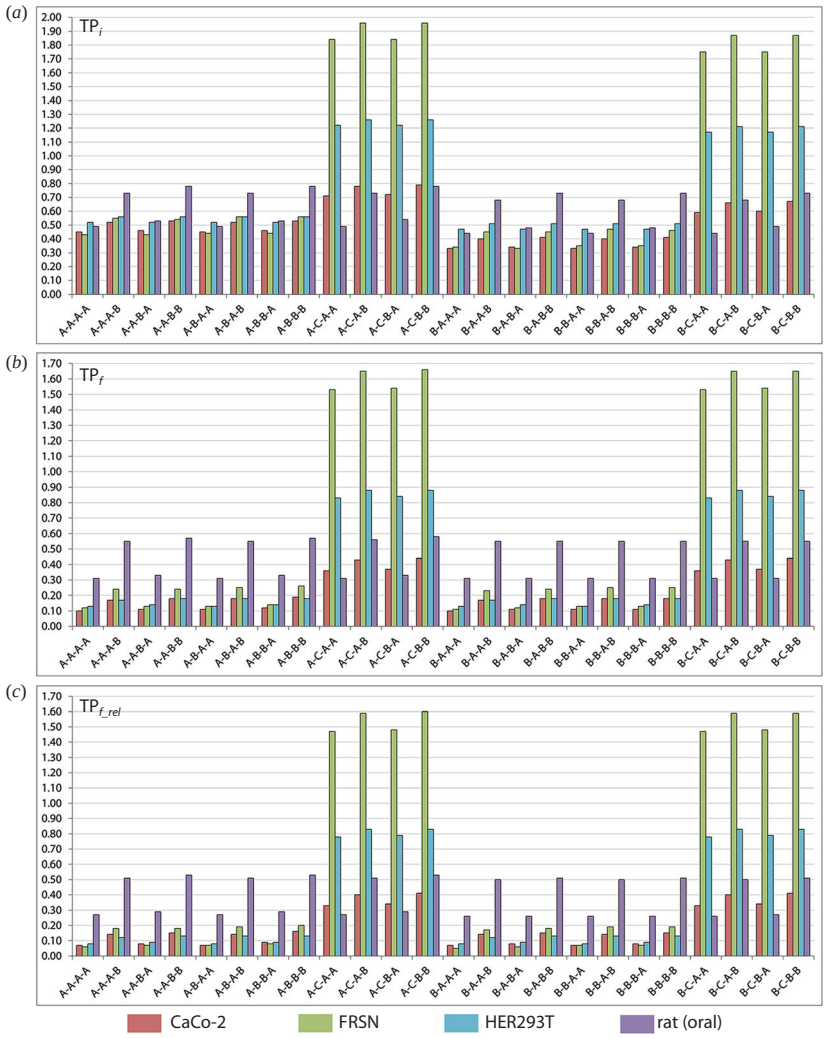

Figure 2 Toxicity potentials (TPs) of 24 routes of synthesis of 1,1′-biphenyl calculated for CaCo-2, FRSN, and HEK293T cells, as well as for rats upon oral administration. The 1st, 2nd, 3rd, and 4th letters in the reaction names correspond to the types of starting material 2 (SM2), catalyst (CT), reagent (R), and solvent (S), respectively (see Table S2 in Online Supplementary Materials). TP_i, initial toxicity potential; TP_f, final toxicity potential; TP_{f_rel}, relative final toxicity potential.

(iii) reagents [R: Na_2CO_3 (A) or K_2CO_3 (B)], and (iv) solvents [S: ethanol (A) or *N*-methylpyrrolidone (NMP, B)]. Figure 1 shows the synthetic routes with the lowest 'overall toxicity', as established in the biological objects used. Bio-Strips for all 24 reactions are shown in Figures S1 (CaCo-2 cells), S2 (FRSN cells), S3 (HEK293T cells), and S4 (rats) in Online Supplementary Materials. Comparisons of TPs are shown in Figure 2 (the exact values are given in Table S2). Of note, BFs of all the reactions analyzed are below 1 in all the biological objects tested which reflects a decrease in the 'overall toxicity' of all the synthetic routes.

According to Figure 1, the analysis carried out using the 24-h CC_{50} values produces four top 'safe variants' of 1,1′-biphenyl synthesis, whereas the analysis using the LD_{50} values in rats suggests three 'safe variants' (see also Table S2). Of them, two variants are the same in all the biological objects studied: the reactions using bromobenzene (B) as SM2, $Pd(OAc)_2$ (A) or $PdCl_2$ (B) as CT, Na_2CO_3 (A) as R, and ethanol (A) as S (reactions B-A-A-A and B-B-A-A).

The most prominent difference between the analyses based on cytotoxicity and acute toxicity values lies in the catalysts: whereas $Pd(OAc)_2$, $PdCl_2$, and especially $Pd(acac)_2$ demonstrate the highest cytotoxicity among the substances tested, their acute toxicity in rats upon oral administration is significantly lower, from *ca.* 7 to *ca.* 23 mmol kg^{-1} b.w. (see Figure 3 and Table S1). In combination with the low quantities of these compounds used in the reaction, the catalysts make insignificant contributions into the 'overall toxicity' and can be selected freely (see Figure 1).

In the case of starting material 2, all the models suggest preferring bromobenzene (B) to iodobenzene (A). This result is related to two observations: first, bromobenzene demonstrates lower toxicity than iodobenzene in all the biological objects studied (though in CaCo-2 and FRSN cells the difference is statistically insignificant), and second, the bromides or iodides produced as byproducts in the reaction also differ in their toxicity. Thus, KBr is less toxic than KI, whereas NaBr shows lower or similar toxicity in comparison with NaI. The impacts of all these substances are reflected by TP_i, TP_f and TP_{f_rel} values of the corresponding reactions (see Figure 2 and Table S2).

The choice of the reagent [Na_2CO_3 (A) or K_2CO_3 (B)] is inessential in accordance with the cytotoxicity data used, whereas the acute toxicity data suggest Na_2CO_3 (A) as a preferable variant because of its lower oral toxicity in rats (see Figure 3).

As for the solvent, all the models point out ethanol (A) as a beneficial variant. Indeed, though NMP demonstrates moderate toxicity in all the biological objects tested (see Figure 3), the high amount of this compound used in the reaction renders it a potential source of harmful environmental impact.

Finally, upon looking at a comparison of toxicity values (24-h CC_{50} and LD_{50}) measured in three cell lines of different origins [CaCo-2 (colorectal adenocarcinoma), HEK293T (human embryonic kidney), and FRSN (mesenchymal stem cells from foreskin)] and in rats upon single oral administration, it can be seen that, apart from the above-discussed differences in the toxicity of $Pd(OAc)_2$, $PdCl_2$ and $Pd(acac)_2$, the cell cultures and rats also differ in their sensitivity to $Na_2B_4O_7$ and $K_2B_4O_7$, which are produced as byproducts in the reactions studied. Still, since these two salts demonstrate similar acute toxicity, their effect on the 'overall toxicity' is insignificant.

Thus, according to the results of our analysis, the bio-Strips and toxicity potentials calculated on the basis of cytotoxicity data produce essentially the same results as the bio-Strips and toxicity potentials on the basis of acute toxicity studied in rats upon oral administration. The only prominent exception is the palladium salts used as catalysts, and in this case, CC_{50}-based analysis marks them as significantly more harmful than LD_{50}-based analysis which is acceptable since in terms of toxicity, it is always better safe than sorry. Of course, it should be remembered that these suggestions are valid for the above-discussed reactions only, and more studies are demanded for making more general conclusions on the topic.

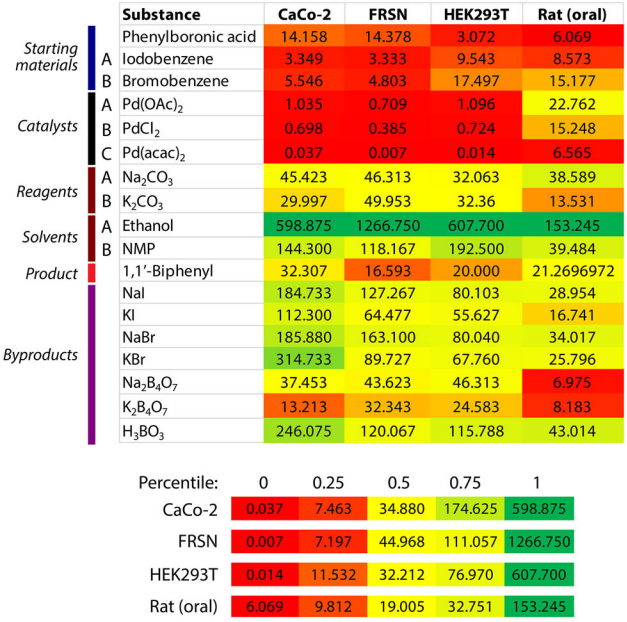

Figure 3 Comparison of the cytotoxicity and acute oral toxicity of the studied substances measured in CaCo-2, FRSN and HEK293T cells, and in rats upon oral administration. The color of the cells matches the 24-h CC_{50} or LD_{50} values provided in the cells (see the legends below the heat map).

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2024.04.013.

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