Predictive Toxicology and Mixtures of Chemicals.

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Abstract

All predictive toxicological models concern individual chemicals. An exposure to a mixture of chemicals is the reality, however. Toxicity of an individual chemical itself or in a mixture with other chemicals may significantly differ. The presentation shows our attempt to find a suitable model for a prediction of acute toxicity of binary mixtures of organic chemicals. We have suggested a molar ratio as a descriptor of the mixture composition and R-plot for a graphical representation of the relationship between acute toxicity and the mixture composition (QCAR – Quantitative Composition – Activity Relationship). The approach was inspired by Rault and Dalton laws, their positive and negative deviations in a mixture behavior of real gases, Loewe and Muschnek isoboles and Finney test of additivity. The inhibition of movement of worms *Tubifex tubifex* has been measured as the acute toxicity with the mixtures benzene - aniline, benzene - ethanol and benzene – nitrobenzene as representatives of the three main types of interactions: potentiation, inhibition, addition. As a physicochemical partner, distribution of components of the mixture between its gaseous and liquid phases has been chosen.. All the relationships should be express in a mathematical function to allow a prediction knowing a composition of a mixture and physicochemical properties of the components.

Introduction.

Toxicity testing is a subject of priority importance to all communities regardless whether scientific, commercial, ecological, regulatory or others. Toxic activities of chemicals on humans or environment have traditionally been tested with a single chemical at a time. To deal with a chemical singly is more convenient and without experience enough the only possibility.

The environment including people is seldom exposed to a single chemical. Contaminated drinking waters, compounds from hazardous waste sites, life style, individual diet or exposure at work places involve an exposure to hundreds of chemicals. Simultaneous or sequential exposures to two or more chemicals can change the toxicity of each chemical both quantitatively and qualitatively. The same is valid for physicochemical properties of single chemicals used in predictive models.

There have been naturally many attempts to predict the joint toxic effects of mixtures of chemicals. The necessity to solve the problem of mixtures toxicity was pointed out by both scientific and regulating authorities. It was reviewed in another places (e.g. Tichý et al. 1998, Tichý et al. 1999). Many models have been developed, mostly without connection with the QSAR analysis. The situation becomes complicated as soon as toxic indices like EC50 or LD50 are considered.

The purpose of this contribution is to add another support to our suggestion of the QCAS (Quantitative Composition – Activity Relationships) analysis (Tichý et al. 1998, Tichý et al. 1999). The aim is a use of a formal principle of the QSAR analysis to predict a toxic activity from the knowledge of physicochemical properties of components of binary mixture of organic chemicals and, in this case, of composition of the mixture.

The second goal is easier and has been described in the papers cited above. Molar ratio of components in the mixture is used as a descriptor of the composition. Relationships between acute toxicity and the molar ratio is expressed in a normalised polynomial function. R-plot demonstrates this relationships in a graphical form (Tichý et al. 1998).

An attempt to find a suitable physicochemical property for the simulation is presented. The attempt is connected with distribution of chemicals, being in their binary mixture, between their gaseous and liquid phases in an equilibrium (Tichý et al. 1999).

In a continuation, concentrations (or amounts) of components in the gaseous phase above a mixture are considered. This index is possible to normalise in respect to that of single chemicals and, thus, to approach the original suggestion used already for the acute toxicity composition simulation.

Experimental.

Acute toxicity was chosen as the toxic effect under the consideration. The acute toxicity was determined as EC50 of the inhibition of movement of worms *Tubifex tubifex*. The method has been described in a standardized manner (Tichý and Rucki 1996, Tichý et al. 1998).

The gas chromatographic measurements were used to determine a composition of the gaseous phase over the binary mixture of organic chemicals chosen and to check the composition and purity of liquid phases. The liquids were mixed in a chosen ratio in a vial, which was tightly closed and the mixture was equilibrated at 25°C.

The gas chromatograph UNICAM PU 4600 with FID detector, digital pressure regulator and capillary column CHROMPACK 10m x 0.32mm with 100% polydimethyl-

siloxane were used. The variation range of measurements was too wide, however, for the low volatile compounds like nitrobenzene.

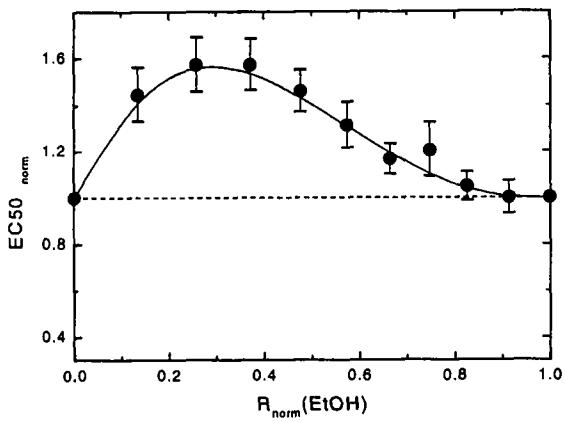

Fig. 1. R-plot of normalised acute toxicity $EC50_{norm}$ for the binary mixture benzene – ethanol representing inhibition of the activities of the components.

Three binary mixtures of organic compounds representing three main types of interactions were chosen: benzene – ethanol representing inhibition of activities, benzene - aniline representing potentiation of activities and benzene – nitrobenzene representing additivity of the activities (Tichý et al. 1999). The R-plots (it means a plot

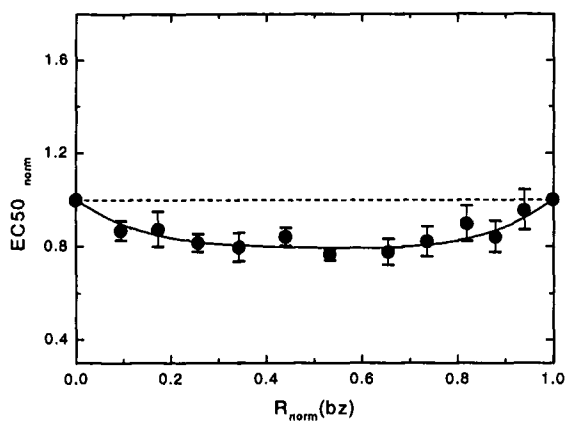

Fig. 2. R-plot of normalised acute toxicity $EC50_{norm}$ for the binary mixture benzene – aniline representing potentiation of the activities of the components.

of normalized acute toxicity $EC50_{norm}$ against the normalized molar ratio R_{norm}(compound)) for the acute toxicity of the three mixtures are shown to demonstrate the differences between the three types of the interaction (Fig. 1 – 3.) The data for the plots were determined once more and proved reproducibility of the determination. They are virtually identical with those measured previously (Tichý et al.1999).

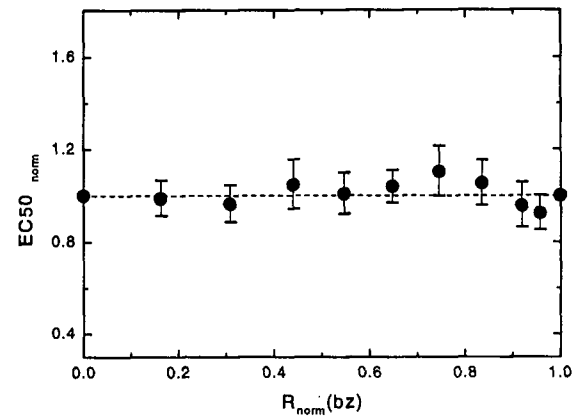

Fig. 3. R-plot of normalised acute toxicity $EC50_{norm}$for the binary mixture benzene – nitrobenzene representing additivity of the activities of the components.

Calculations and graphical demonstrations

(Tichý et al 1998a).

The molar ratio R_a was defined as a ratio between concentration c of a compound a to a sum of concentrations of both compounds in a mixture a + b:

$$R_a = c_a/(c_a + c_b).$$

The normalized EC50 were obtained by a normalization in respect to the EC50 of a pure compound like:

$$EC50(a)_{norm} = EC50(a)_{mixture}/EC50(a)_{pure}.$$

$$EC50_{norm} = EC50(a)_{norm} + EC50(b)_{norm}$$

So that $EC50(a)_{norm}$ of the pure compound *a* equals 1 and $EC50(b)_{norm}$ of the pure compound *b* equals 1 as well.

The normalized molar ratio of a component *a* is then related to the concentration $EC50(a)_{pure}$ (or $EC50(b)_{pure}$) as well:

$$R_{norm}(a) = [c_a/EC50(a)_{pure}]/[(c_a/EC50(a)_{pure}) + (c_b/EC50(b)_{pure})]$$

The additivity is expressed in the R-plot as a horizontal line connecting the points <0, 1> and <1, 1>. The deviations from the additivity can be noticed as deviations from the additivity line upwards (inhibition) or downwards (potentiation).

Results and Discussion.

The usage of the gas chromatographic analysis of the gaseous phase over the liquid one of the same binary mixture at the equilibrium is shown in the plots log (R_g/R_l) vs. log R_l (Figs. 4 – 6) and the plots $(\Sigma n_i)_{norm}$ vs. R_l (Figs. 7 – 9).

The molar ratio of a component in the gaseous or corresponding liquid is defined by the same way as shown above for the R-plots. The plots for both components is always given in the same figure, the identification of an appropriate compound to a plot being shown directly in the figure.

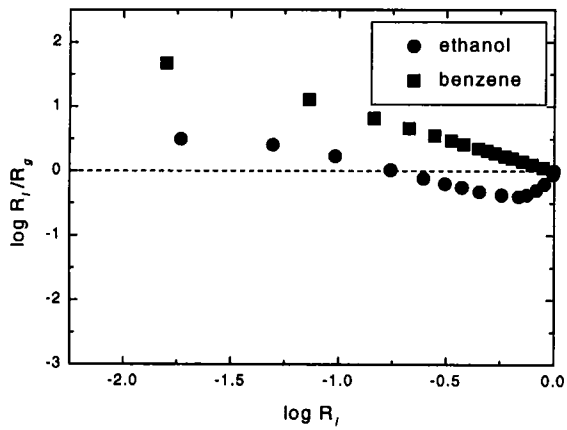

Fig. 4. Benzene . ethanol mixture. A relationship between the composition of gaseous and liquid phases expressed in molar ratio of their components.

Three types of the plots can be seen:

- the curve-like branch is crossing the horizontal line passing through zero (Fig. 4, benzene – ethanol mixture, inhibition)
- the curve-like branch does not cross the "0" line, but forms "a close" shape of the graph (Fig. 5, benzene – aniline mixture, potentiation)
- the curve-like branch does not cross the "0" line and forms rather "open" shape of the graph (Fig. 6, benzene – nitrobenzene mixture, additivity).

An attempt to use a property which can be separated for the individual components of the mixture followed.

The values of amount n_i (concentration) of the individual components (i) in the gaseous phase above the mixture were determined. In the case of low volatile compounds like nitrobenzene or at the lowest concentrations of components in the phase, very sensitive and careful measurements are necessary. For semiquantitative demonstration

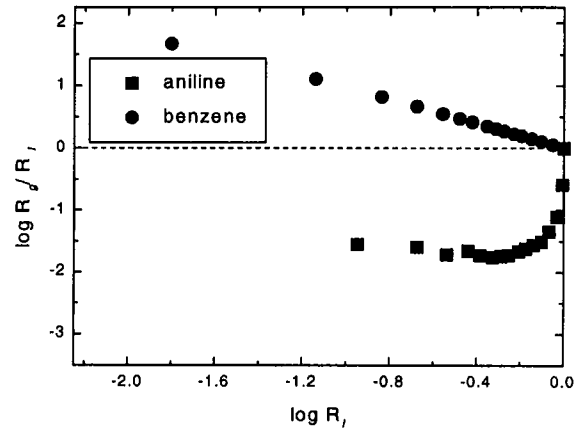

Fig. 5. Benzene – aniline mixture. A relationship between the composition of gaseous and liquid phases expressed in molar ratios of their components.

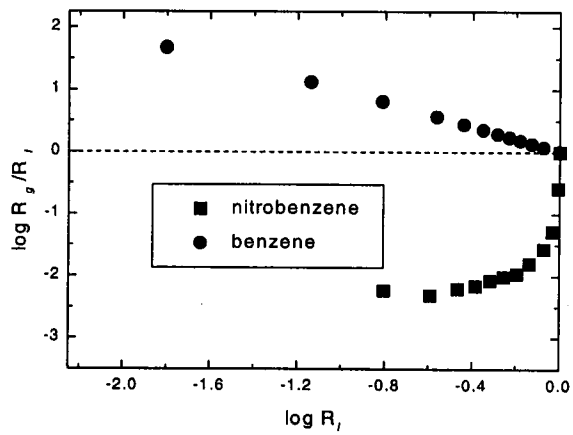

Fig. 6. Benzene – nitrobenzene. A relationship between the composition of gaseous and liquid phases expressed in molar ratios of their components.

the already made measurements remains useful (Figs. 7 – 9), however.

The normalization of the values Σn_i was done by a similar way as that of EC50, ie.

$$(\Sigma n_i)_{norm} = [n_a(\text{mixture})/n_a(\text{pure})] + [n_b(\text{mixture})/n_b(\text{pure})]$$

The molar ratio R_l was calculated in the same way as in the previous cases.

The plots for all measured compositions of the mixtures reflected:

- the inhibition (Fig. 7): all points are above the zero line regardless their position which cannot be interpreted yet,

- the potentiation (Fig. 8): All points are below the zero line,
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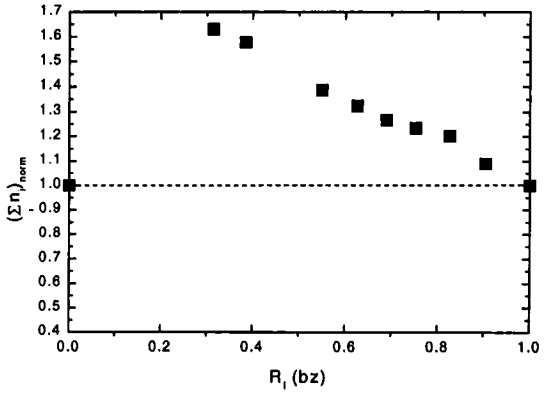

Fig. 7. Benzene – ethanol mixture. Normalized total amount $(\Sigma n_i)_{norm}$ of both components (i) in the gaseous phase vs. molar ratio of benzene in the liquid phase R_l(bz).

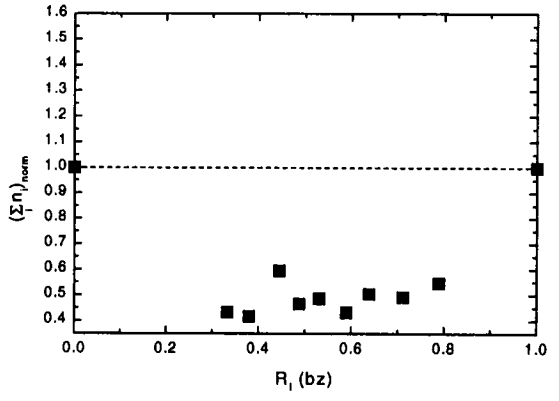

Fig. 8. Benzene – aniline mixture. Normalized total amount of both components in the gaseous phase over the mixture vs. molar ratio of benzene in the liquid phase.

- the additivity (Fig. 9): the points are placed on the line connecting the amount of nitrobenzene in its gaseous phase and that of benzene in the gaseous phase above the pure liquids (there were not data enough for the normalization).

To solve a prediction of toxicity of chemicals in their mixtures remains a problem for the future. To find quantitative relationships with the behavior of physicochemical properties of chemicals under real conditions if in mixtures could be a topic where AI may significantly help. The results indicate that a parallel might exist between changes in acute toxicity and changes in physicochemical properties of chemicals if in mixtures and act jointly. The precedent exists in the relationships between hydrophobi-

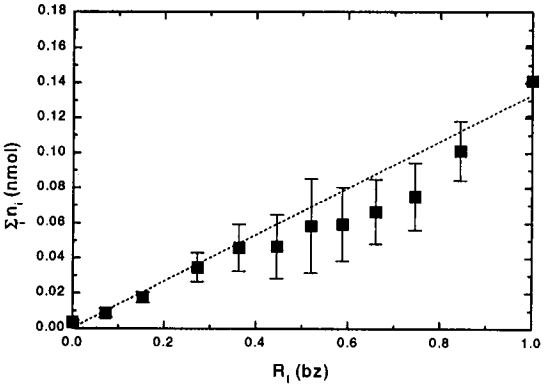

Fig. 9. Benzene – nitrobenzene mixture. The total amount Σn_i (nmol) (normal scale, non-normalized) of both components in the gaseous phase of the mixture vs. molar ratio of benzene in the liquid phase R_l(bz).

ty of individual chemicals and their biological activities (QSAR).

Acknowledgements.

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