



2-FUN

*Full-chain and **UN**certainty Approaches for Assessing Health Risks in
FUture ENvironmental Scenarios*

**FP6 Project-2005-Global-4
Integrated Project - Contract n°: 036976**

– DEMONSTRATOR FOR THE METHODOLOGY, WITH THE COUPLING OF PBPk AND PATHOLOGY MODELS –

Due date of delivery: *01/08/2008*

Actual submission date: *06/10/2008*

Start date of the project: *01/02/2007*

Duration: *48 Months*

Lead contractor organisation name for this deliverable: JRC

Project co-funded by the European Commission within the Sixth Framework Programme (2002-2006)	
Dissemination Level	
PP	Restricted to other programme participants (including the Commission Services)

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Document Information

Document Name Demonstrator for the methodology, with the coupling of PBPK and pathology models
ID D3.2_demonstrator_vf.doc
Revision FinalVersion
Revision Date 31/08/2008
Author A. GOTTI / JRC
Co-Authors D.SARIGIANNIS / JRC

Approvals

	Name	Company	Date	Visa
Author	A. GOTTI	JRC	22/09/2008	A. Gotti
Co-Author	D. SARIGIANNIS	JRC	22/09/2008	D. Sarigiannis
WP Leader	C. BROCHOT	INERIS	26/09/2008	C Brochot
Coordinator	F. BOIS	INERIS	06/10/2008	F. Bois

Documents history

Revision	Date	Modification	Author
Version 1	31/08/2008	First version	A. Gotti
Version 2	22/09/2008	Modification of the structure: introduction, conclusion.	A. Gotti
Version 3	26/09/2008	Minor modifications (administrative information)	C. Brochot



Introduction

Among the four VOCs considered in this work (benzene, toluene, xylene and ethylbenzene) benzene represents certainly the most potentially dangerous to human health. Chronic exposure to low levels of benzene may produce reversible decreases in blood cell numbers but, at higher levels, an irreversible bone marrow depression, with pancytopenia, may establish. This pathological condition is called aplastic anemia. Pancytopenia can occur also in the so-called myelodysplastic syndrome (MDS). Benzene MDS usually proceeds to leukaemia, mostly acute myeloid leukaemia (AML) and it has then been found to induce many different kinds of genetic damage, such as point mutations, DNA adducts, oxidative DNA damage, structural and numerical CA, MN, cell transformation, in a variety of *in vitro* and *in vivo* systems.

Numerous review articles can be found in the open literature that discuss benzene carcinogenesis (e.g., Aksoy [1]; Cox 1991 [2]; Gold et al. [3]; Snyder and Kalf [4]; Snyder et al.[5]). In AML, there is diminished production of normal erythrocytes, granulocytes, and platelets, which leads to death by anemia, infection, or hemorrhage. The hallmark of AML is the appearance in the peripheral blood of cells morphologically indistinguishable from myeloblasts. These events can be rapid. Case reports and epidemiological studies of workers have established a causal relationship between benzene exposure and AML (Crump [6]; Infante [7]; Infante et al. [8]; Paxton et al., [9], [10]; Rinsky et al.[11]; Wong [12], Wong and Raabe 1995 [13]).

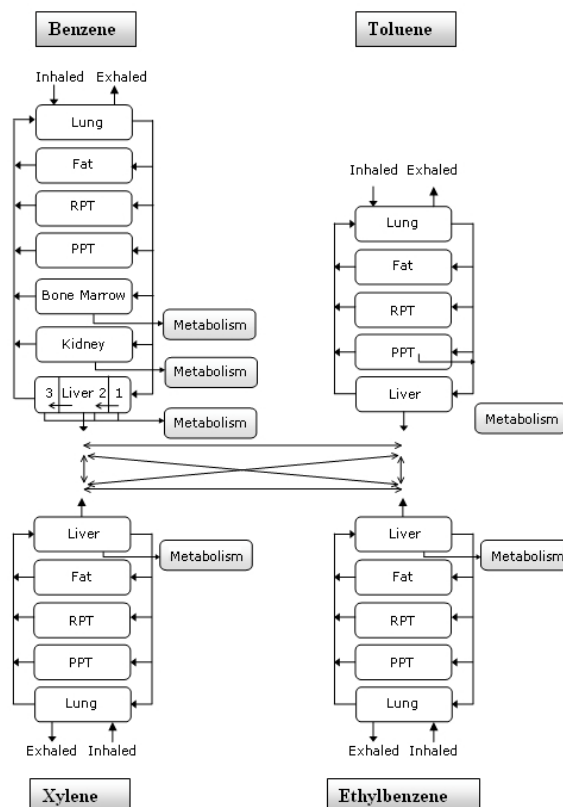
Toxicokinetics and metabolism model

Out of the volatile organic chemicals the ones with a distinct presence in all types of indoor environments, are benzene (B), toluene (T), ethylbenzene (E) and o-, m-, p-xylene (X) (here all three xylene isomers will be taken as the xylene group). The so-called BTEX mixture was chosen as a good model for studying the efficacy and refining the multi-layer integrated approach outlined in this work since benzene has been accused of carcinogenic properties (especially when attacking the bone marrow resulting thus in leukemia), neurotoxicity and co-exposure with other aromatic compounds has been suggested to alter the rate of benzene metabolism at the liver and the bone marrow (about 10% of benzene is metabolized at the bone marrow). Given that the main metabolites of benzene are more potent carcinogens than the parent compound, the biochemical interaction of the four aromatic substances at the level of metabolism may have profound influence on the toxic potency of the overall chemical mixture. It has been theorized that these interactions are dose-dependent; therefore it is of the essence to consider how health risk from this mixture is affected by biochemical interaction at the dose levels the public at large is generally exposed to. These levels are generally much lower than the ones found in occupational settings (e.g. in the tire, rubber, paint and shoemaking industries); the unit risk for the substances in the mixture, however, is calculated by WHO expert groups on the basis of epidemiological data, which are consistently based on higher, occupationally-relevant levels of exposure. Thus, the actual public health risk from environmental exposure to this type of VOC mixture needs to be revised on the basis of a more comprehensive assessment of the biological and physiological responses to environmentally relevant doses of the constituting substances.



The sub-model developed for benzene is a six-compartment model that is an extension of that of Medinsky et al. (1989) [14] and Travis et al. (1990) [15] adding the kidney as a further site of metabolism. The six tissue groups utilized in the model include (1) the liver (main metabolic tissue); (2) fat tissue; (3) bone marrow; (4) richly perfused tissues (RTP), (5) poorly perfused tissues (PPT) and (6) the kidney; each one is interconnected to the others by systemic circulation and a gas-exchange lung. The kidney was included as a tissue compartment since it is recognized to be a potential site of benzene metabolism. Benzene was assumed to be eliminated by exhalation or through Michaelis-Menten metabolism in the liver, and to a lesser extent, in the kidney and in the bone marrow. To better describe benzene metabolism the liver was further subdivided into three compartments of equal volume, which represent three zones of the liver where specific enzymes allowing enzyme-mediated metabolism are found. In particular the metabolic transformations mediated by CYP2E1 are supposed to occur in zone 3 of the liver, sulfation takes place primarily in zone 1 of the liver while non-enzymatic metabolism occurs in all the three compartments.

The three-sub-models for toluene, xylenes and ethylbenzene are all four-compartment models similar to that of Tardif et al (1997) [16] and of Haddad et al. (1999) [17]. Metabolism is assumed to occur only in the liver but no further subdivision of it was considered necessary. The overall conceptual representation of the PBPK/PD model developed for the mixture of BTEX is depicted in the following figure.





The metabolic chain of benzene was modeled starting from previously developed PBPK/PD models for benzene metabolism in mice (Medinsky et al, 1989) [14] and its extrapolation to humans (Tardif et al, 1997) [16]. The model evaluates tissue levels of benzene, benzene oxide (BO), phenol (PH), and hydroquinone (HQ), as well as the total amounts of muconic acid (MA), phenylmercapturic acid (PMA), phenol conjugates, hydroquinone conjugates, and total catechol and trihydroxy benzene (plus conjugates) produced. For benzene oxide, phenol, and hydroquinone, the body is divided into five compartments: kidney; liver; fat; rapidly perfused tissues, which consist of brain, heart, spleen, intestines, and bone marrow; and slowly perfused tissues, which contain muscle and skin. As for the benzene model the liver is subdivided into three compartments of equal volume according to the specific enzymatic distribution. Further metabolism of BO, PH and HQ is supposed to occur in the liver (main metabolism site) and to a lesser extent in the kidney. The detailed mathematical models describing both the biochemical interactions leading to metabolic inhibition of benzene due to the presence of the other antagonistic VOCs in the BTEX mixture and the kinetics of the BTEX distribution in the human body are given in report D.3.1. These models are now fully implemented in the 2-FUN demonstrator software based on the ECOLEGO motor.

Pathology model

Currently, there are four main occupational cohort studies demonstrating an association between benzene and leukaemia and for which exposures have been assessed in detail. These are the Goodyear Pliofilm (Crump [6], Rinsky et al., [18], [11] the Chemical Manufacturers Association (CMA) [12], [19], Dow Chemical and the Chinese Factory Worker cohorts [6]. WHO (2000) [19] decided to use the study of Crump (1994) on the Goodyear Pliofilm cohort to derive a quantitative cancer risk estimation associated to benzene inhalation. They estimated a range of 4.4×10^{-6} to 7.5×10^{-6} with a geometric mean of 6.0×10^{-6} as the increase in the lifetime risk of an individual who is exposed for a lifetime to $1 \mu\text{g}/\text{m}^3$ benzene in air. Human cancer risk from benzene exposure estimated from these studies is based primarily on epidemiological data with supporting evidence provided by animal bioassay data. The common approach links external exposure to the probability of developing cancer through the definition of a dose-response curve.

The risk assessment approach outlined above has two main limitations: first it ignores the uncertainty associated with external exposure as well as variability between different individuals (inter-individual variability) stemming from genetic differences, lifestyle, age or physiological status; all of the above may result in differences in actual health outcomes. Second, the main dose metric used to relate the administered dose history to cancer probability is the AUC (Area Under the Curve). This means that the same dose (and hence the same risk) is assigned to any dose history having the same integrated total dose without regard to the time evolution of the administered dose.

Since the cancer risk associated to benzene is mainly related to its metabolites [21], [22] and, more in detail, to their internal dose, a more biology-based approach that takes into account the internal dose of metabolites produced should represent an improvement in the traditional risk assessment giving also more robust biological basis. Furthermore the use of PBPK/PD models allows to take into account the uncertainty associated to inter-



individual variability through the application of Monte-Carlo Markov Chain techniques providing a distribution function of health risk to the exposed population rather than a unique value representing an “individual” risk.

The cancer risk model developed in this work, follows the approach of Cox [23]; it is based on the decomposition of the dose-response relation into two distinct sub-relations: the first one links the administered dose to the total amount of benzene metabolites produced (internal dose) while the second one connects the internal dose to the cancer probability through an empirical-statistical model.

The first sub-relation is provided by the PBPK/PD model. It allows to quantify the total benzene metabolites produced per day and kilogram of body weight due to a whatever external dose.

A detailed description of the PBPK/PD model for benzene and its metabolic chain is included in the 2-FUN deliverable D3.1 “Detailed description of the general PBPK model, describing metabolic interactions”.

The application of the first step results in the table 1 showed hereinafter:

Table 1: Human Internal doses of benzene metabolites produced from different inhalation exposure

	Concentration (ppm)						
	1	5	10	25	50	100	200
Average daily internal dose of metabolites (mg/kg/day) from PBPK/PD model	0.072	0.355	0.694	1.616	2.844	4.492	6.386

With regard to the second sub-relation, the main assumption according with Crump and Allen [24] and with Bailer and Hoel [25] was that equal internal dose of benzene metabolites produces equal cancer risk in humans and animal; in other words they assumed the same internal dose-response functions for humans and mice.

In their work Crump and Allen estimated the quantitative cancer risk to human populations exposed to benzene based on both epidemiological data and animal bioassay data. The risk estimate was obtained by fitting an LMS model to the oral gavage dose-response data for male mice reproduced in table 2.

Table 2: Administered dose-response data for Male B6C3F1 Mice.

Administered dose (mg/kg/day)	0	25	50	100
Internal doses (mg/kg/day)	0	18	29	42
% of mice with cell carcinomas	0	8	40	74



This last table allows to derive an empirical-statistical relation that links the internal doses to the cancer probability. The latter was found to be equal to:

$$\text{Cancer probability for } x \text{ mg/kg/day} = 1 - e^{-(0.00145x+0.00013x^2)} \quad [1]$$

where x represents the dose administered to the mice.

Joining the data contained in the two above tables we derived a new empirical-statistical relation that allow to estimate the cancer risk for humans starting from the internal doses of total benzene metabolites (y).

The latter was found to be described by the following non-linear regression model:

$$P(y) = 1 - e^{[-0.04296940y+0.02633730y^2-0.00764081y^3]} \quad [2]$$

with a residual standard error of 0.00858475 on 8 degrees of freedom.

The estimated cancer risk as a function of the external benzene concentration as predicted by the above function is represented in Figure 1 by the dashed line. For comparison, in the same figure is also represented the Crump and Allen model results (solid line).

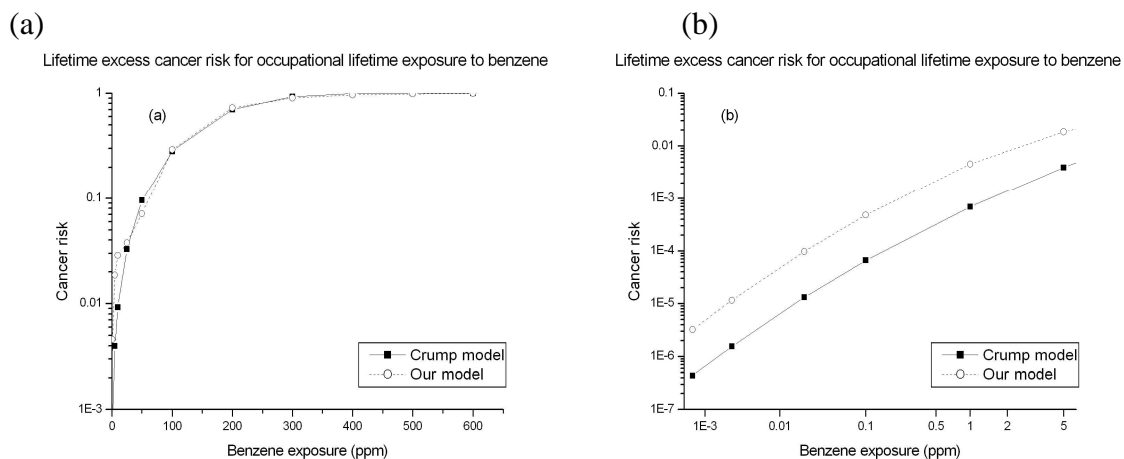


Figure 1: (a) lifetime excess cancer risk for 8hr/day lifetime inhalation exposure to benzene as predicted by Crump and Allen and our model. (b) the same at low doses.

Conclusions

The coupled biokinetics-metabolism-pathology model described above provides a complete tool for the assessment of carcinogenicity of similar chemical compounds such as aromatic VOCs found at environmentally relevant levels in the ambient and indoor air across Europe. The kinetics and metabolic models have already been implemented in the 2-FUN demonstrator; the pathology model will be implemented in the demonstrator



software in October-November 2008. This modelling framework provides reliable estimates of risk of leukaemia from chronic co-exposure to the BTEX mixture and to other structurally similar chemical compounds on the basis of mechanistic information regarding the biochemical interactions among the mixture components. Once implemented fully on the 2-FUN demonstrator, it will allow the application of a Markov chain Monte Carlo procedure based on Bayesian statistics to provide population cancer risk estimates in terms of probability distribution functions following the 2-FUN paradigm for uncertainty management in risk assessment.

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