Health risk from accidental fire in a plastics recycling facility

D.A. Sarigiannis*,**, S.K. Karakitsios* , A. Gotti**

* *Aristotle University of Thessaloniki, Department of Chemical Engineering, Environmental Engineering Laboratory, University Campus, 54124, Thessaloniki, Greece, denis@eng.auth.gr*

** *Environmental Health Engineering, Institute for Advanced Study, Piazza Della Vittoria, 15, 27100, Pavia, Italy*

Corresponding author: Prof. Dimosthenis Sarigiannis, denis@auth.eng.gr, telephone number: 2310994562

Abstract

Purpose: Waste recycling facilities may be associated to adverse health outcomes in the aftermath of industrial accidents involving the inadvertent generation of toxic chemicals and their release into the environment. The study aims at calculating the health burden (in terms of cancer risk) of the population living in the Aspropyrgos area (Greece) due to increased exposure to dioxins and furans (PCDDs/PCDFs), emitted by an accidental fire in a plastics recycling plant.

Methodology: To properly account for the additional internal exposure burden resulting from the accidental event, the generic Physiology Based Bio-Kinetic (PBBK) model of the computational platform INTEGRA was used.

Result: Analysis of ambient air samples (both particle and gaseous phase) showed that the levels of PCDDs/PCDFs in the surrounding area were 1.8 pg/m^3 TEQ WHO (toxicity equivalent concentration in accordance with the methodology of the World Health Organization), which are considered significantly higher than the 0.1 pg/m^3 TEQ WHO, reported as background levels. This additional exposure was translated into an internal exposure and finally into a new equivalent chronic uptake dose, estimated equal to 0.0023 pg_TEQ/kg_bw/d. For the population exposed over 6 days to the PCDDs/PCDFs fumes emitted from the recycling plant fire the respective risk was up to $2.91 \cdot 10^{-7}$, which is 13% higher than the background level of risk in a 30-year period.

Conclusion: Although waste recycling is considered one of the most environment friendly and sustainable waste management option, accidental events might result in significant contamination of the surrounding area, affecting adversely the population leaving nearby.

Keywords: plastics, recycling, dioxins, furans, cancer risk.

1 Introduction

Waste recycling is one of the main cornerstones of the EU waste management strategy [1]. It offers many advantages contributing to the circular economy and the sustainable and efficient use of natural and man-made resources. However, waste recycling facilities may be associated to adverse health outcomes in the aftermath of industrial accidents involving the inadvertent generation of toxic chemicals and their release into the environment.

One of the major concerns associated to accident in plastic recycling plants are the emissions of dioxins and furans (PCDDs/PCDFs). These compounds are characterized by a high carcinogenic potency [2]. Because PCDDs/PCDFs appears to be acting like a potent and persistent hormone agonist, it appears reasonable to incorporate mechanistic information on receptor-mediated events in risk assessments for TCDD. This information may be obtained from steroid receptor action and from molecular data on the Ah receptor [3]. This receptor based toxicity, results in sex-dependent sensitivities, as a result of a set of sex-specific PCDDs/PCDFs -responsive genes. However, the estimation of the additional probability of cancer due to the additional exposure burden is quite difficult [4]. A major obstacle is that an elevated short term external exposure associated to the accidental event, has to be translated into long term risk estimates. Considering the significant persistency and bioaccumulation of PCDDs/PCDFs in human body, the use of biokinetic models for assessing the actual internal dosimetry of this complex mixture is of particular importance. The pharmacokinetics of TCDD are relatively well understood in adult humans [5-7]. However, the impact of pregnancy and lactation on the elimination of TCDD and other dioxins is not clear [8]. Additional insides regarding the biological perturbations induced by PCDDs/PCDFs exposure are provided by transcriptomics and metabolomics analysis, where

altered levels of endogenous steroid metabolites and modified urinary bile acids profiles were identified upon acute exposure to PCDDs/PCDFs [9]. Taken together, these findings are compatible with an increased expression of cytochrome P450s, persistent hepatotoxicity, bile acid homeostasis dysregulation and oxidative stress. In *in vivo* studies (mice), serum metabolomics identified azelaic acid monoesters as significantly increased metabolites after TCDD treatment, due to downregulation of hepatic carboxylesterase 3 (CES3, also known as triglyceride hydrolase) expression in an arylhydrocarbon receptor (AhR)-dependent manner [10]. The decreased CES3 expression was accomplished by TCDD-stimulated TGFβ-SMAD3 and IL6-STAT3 signaling, but not by direct AhR signaling, indicating that PCDDs/PCDFs affect additional pathways beyond the ones regulated by AhR. With regard to AhR deregulation, PCDDs/PCDFs exposure elicited metabolite and gene expression changes *in vivo* subjects (mice) associated with lipid metabolism and transport, choline metabolism, bile acid metabolism, glycolysis, and glycerophospholipid metabolism [11]. From transcriptome analysis of mitogen-induced lymphocytes cultured with 10 nM TCDD, all AhR-dependent genes were induced 1.2- to 13-fold and plasma TCDD was associated with decreased 7-ethoxyresorufin O-deethylase activity, as well as strong positive correlation between AhR and CYP1A1/ CYP1B1 expression [12].

Accidental fires in plastic industry comprise one of the major events resulting in contamination of various environmental media to PCDDs/PCDFs of the surrounding area; air samples collected in the 5th day of the event were found to contain over 1000 pg/m^3 TEQ (toxic equivalent quantity) of dioxin, exceeding background levels by 2,500−25,000 fold [13]. Based on the above, the current study aimed at calculating the health burden (in terms of cancer risk) of the population living in the Aspropyrgos area (close to Athens, Greece) due to increased exposure to dioxins and furans (PCDDs/PCDFs), emitted by an accidental fire in a plastics recycling plant in June 6, 2015. The fire resulted in significant particle and gaseous emissions of several compounds related to plastic industry. In addition, release of dioxins and furans was a major concern, due to their persistence in environmental and biological matrices, as well as to their carcinogenic potency. In order to face the methodological problem mentioned above, a comprehensive methodology involving both measured data and complex internal exposure modelling was employed.

2 Methodology

2.1 Overall study design

In order to estimate the risk related to the PCDDs/PCDFs emitted during the fire, it was critical to estimate the long term internal burden of exposure associated to this event. The need for addressing long term exposure is associated to the fact that PCDDs/PCDFs are bioaccumulative and persistent with a half-life time of almost 7.5 years in humans. Hence, it is critical to translate the actual uptake during the accidental event (that lasted for a few days) into a long term (lasting for many years) internal exposure burden. The only scientifically sound way to translate these external doses into internal exposure to the target tissues was carried out with physiology based biokinetic (PBBK) models. To be able to perform this type of calculation, it was critical to be able to identify (a) the background exposure levels to PCDDs/PCDFs and (b) the additional burden of exposure due to this accidental event. Towards this aim, the following data were needed:

- Data on ambient air levels of PCDDs/PCDFs during the accidental event were obtained by various measurements of PM and analysis of PCDDs/PCDFs in the particle and gaseous phase.

- HBM data for estimating the background exposure to the exposed population. In practice, the HBM data of the local population collected from a previous study (before the accident) where used to estimate the equivalent background exposure that results in the corresponding HBM data; the additional exposure of the measured PCDDs/PCDFs was added to these background levels for a duration of 6 days.

- HBM data of PCDDs/PCDFs in the blood for the population (50 individuals) living in the area after the accidental event, for verifying the increased internal exposure levels.

- Metabolomics analysis (using an aliquot of the blood samples of the 50 individuals) for identifying the perturbed metabolic pathways**.** Various statistical techniques can be combined in a proper way to identify potential biomarkers and demonstrate how useful the selected metabolomics are.

2.2 Applied methodology

2.2.1 Integrated exposure assessment and internal exposure modelling

Realistic exposure assessment is data-intensive, requiring detailed information at every step of the source-to-dose

pathway. Aiming at associating external exposure estimates from short-term short term ambient air contamination with long-term internal dose, the computational platform INTEGRA [14] was used, that aims at bringing together all available information within a coherent methodological framework for assessing the source-to-dose continuum for the entire life cycle of substances covering an extensive chemical space. The major component of INTEGRA is a computational platform that integrates environmental fate, exposure and internal dose dynamically in time. The platform is largely validated using human biomonitoring data from Europe and the USA. Hence, the major component of INTEGRA [14] is an integrative computational platform that comprises environmental fate, exposure and internal dose dynamically in time, including the following components:

1. Development of a multimedia model to account for multi-scale interactions affecting the environmental transport and fate of chemicals. The model takes into account interactions within various scales (e.g. continental, regional and local) and mass exchange among various phases and media (e.g. soil, air water and sediment).

2. Development of indoor micro-environmental modelling and detailed personal exposure assessment, including all potential exposure pathways and routes of exposure. It has to be noted that PCDDs/PCDFs are highly lipophilic compounds and they adsorb strongly on particles. As a result, for properly estimating exposure to PCDDs/PCDFs estimation of actual uptake of particles across human respiratory tract is necessary. HRT particle deposition modeling was applied for the determination of PM deposition fraction (DF) to the three parts of the pulmonary system in order to estimate the uptake of PCDDs/PCDFs. Major mechanisms of PM deposition across HRT include diffusion, sedimentation and impaction. Secondary mechanisms involve interception and electrostatic deposition. Different HRT regions involve different deposition mechanisms, with regard to different PM size as follows:

- Naso-pharyngeal region (or upper respiratory tract – URT): impaction, sedimentation, electrostatic (particles > 1 μm)
- Tracheo-bronchial (TB) region: impaction, sedimentation, diffusion (particles < 1 μm)
- Pulmonary (P) region: sedimentation, diffusion (particles < 0.1 μm)

Several parameters affect HRT deposition, including PM properties (concentration and size distribution), air flow parameters (lung capacity and breathing frequency) and HRT physiology (structure and morphology). All of these parameters have been taken into account in the approach proposed herein.

HRT deposition was carried out using the Multiple Path Particle Deposition (MPPD) v. 2.1 model [15]. Age-specific lung geometries representing 10 distinct ages from 3 months old to 21 years old are also provided. An idealized symmetric single-path model as well as a 5-lobe symmetric multiple-path model are available for use with each age setting [16-18]. Software inputs include morphological parameters of pulmonary system – functional residual volume (FRC), tidal volume (TV), upper respiratory tract (URT) volume, as well as breathing frequency (BF) for each age group

3. Development of a generic PBBK model so as to incorporate life stage changes and physiological and metabolic efficiency change over an individual's lifetime (from conception till 80 years of age). The model is able to cover perinatal exposure including exposure routes such as lactation, being practically a mother-fetus interaction model. Advanced QSAR models are used to estimate physicochemical and biochemical parameters of the model in order to expand its applicability domain to a large chemical space [19]. The fundamentals of PBPK modelling are to identify the principal organs or tissues involved in the disposition of the chemical of interest and to correlate the chemical absorption, distribution, metabolism, and excretion within and among these organs and tissues in an integrated and biologically plausible manner. Within the boundary of the identified compartment (e.g., an organ or tissue or a group of organs or tissues), whatever inflows must be accounted for via whatever outflows or whatever is transformed into something else. This mass balance is expressed as a mathematical equation with appropriate parameters carrying biological significance. A generic equation, for any tissue or organ, is:

$$V_i \frac{dC_{ij}}{dt} = Q_i(CA_j - CV_{ij}) - Metab_{ij} - E\lim_{ij} + Absorp_{ij} - \Pr Binding_j$$

where $\boldsymbol{V_i}$ represents the volume of tissue group $\boldsymbol{i}$, $\boldsymbol{Q_i}$ is the blood flow rate to tissue group $\boldsymbol{i}$, $\boldsymbol{CA_j}$ is the concentration of chemical $\boldsymbol{j}$ in arterial blood, and $\boldsymbol{C_{ij}}$ and $\boldsymbol{CV_{ij}}$ are the concentrations of chemical $\boldsymbol{j}$ in tissue group $\boldsymbol{i}$ and in the effluent venous blood from tissue $\boldsymbol{i}$, respectively. $\boldsymbol{Metab_{ij}}$ is the rate of metabolism for chemical $\boldsymbol{j}$ in tissue group $\boldsymbol{i}$; liver, being the principal organ for metabolism would have significant metabolism and, with some exception, usually $\boldsymbol{Metab_{ij}}$ is equal to zero in other tissue groups. $\boldsymbol{Elim_{ij}}$ represents the rate of elimination from tissue group $\boldsymbol{i}$ (e.g., biliary excretion from the liver), $\boldsymbol{Absorp_{ij}}$ represents uptake of the chemical from dosing (e.g., oral dosing), and $\boldsymbol{PrBinding_{ij}}$ represents protein binding of the chemical in the tissue. All these terms are zero unless there is definitive knowledge that the

particular organ and tissue of interest has such processes. A series of similar mass balance differential equations representing all of the interlinked compartments are formulated to express a mathematical representation, or model, of the biological system. This model can then be used for computer simulation to predict the time course behavior of any given parameter in the model. In the current study, the model was properly parameterized for PCDDs/PCDFs. The biokinetics of TCDD are relatively well understood in adult humans [5,7,6] and several key parameters (tissue partition coeficients and clearance rates) were used from these models. The key aspects characterizing TCDD biokinetics are very high lipophilicity (adipose:tissue blood patrition coefficient is equal to 220) and very slow elimination rate, resulting in a half-life elimination rate of 7.5 years, explaining its long persistance and bioaccumulation potential. Another important issue to be addressed is the transfer of PCDDs/PCDFs through placenta and maternal milk. The model describes mother fetus interactions by modelling the intra-placental properties that govern the transfer of xenobiotics and their metabolites from the mother to the fetus as it grows. The anthropometric parameters of the models are time dependent, so as to provide a lifetime internal dose assessment, as well as to describe the continuously changing physiology of the mother and the developing fetus. The model include the diffusion flow from uterus to placenta and vice-versa during pregnancy [20]. Excretion via lactation is described as an output from the mammary tissue compartment through a partitioning process between mammary tissue and milk, and milk withdrawal by suckling, as described for PCBs in rats [21] and further adopted for humans [22].

4. Inverse modelling for exposure reconstruction and human biomonitoring (HBM) data assimilation. This module, allows the translation of HBM data into external exposure estimates i.e. the exposure that corresponds to the observed biomonitoring data [23]. Based on the detail of the ancillary information, estimates about the contribution of the various pathways and routes can be attributed as well.

2.2.2 Human biosamples, metabolomics and pathway analysis

In a selected number of 50 individuals living in the area, blood sample was obtained for analysis of the PCDDs/PCDFs levels, as well as for metabolomics analysis. Almost 50 ml of blood were collected from each individual and the samples were immediately processed for serum separation. The analytical procedure followed the CDC protocol (without using the FMS-system) and method 8290, while all the results were lipid adjusted [24]. Analysis of blood samples was carried out in an Agilent high-resolution gas chromatography/mass spectrometry (GC/MS).

300 μl blood serum was transferred to 1.5 ml small eppendorf. At the same time, 50μl of each sample was placed in a falcon-type container for the preparation of the Quality Control QC sample and stored at -80 ^{o}C. A three-fold amount of ice-cold solvent, in this case 900 μl of methanol, was added to precipitate the sample proteins in the form of a white precipitate. These aliquots were centrifuged for 20 minutes in a standard centrifuge at 10,000 rpm. After completion of centrifugation, 950 μl of the supernatant was taken and placed in 1.50 ml small eppendorf and stored at -80 °C. When moving forward with the analysis, the samples were centrifuged for 5 minutes at 10,000rpm, because residue was observed at the bottom. 500 μl,were taken, using a special speed-vac device, evaporated and dried with N_2. The same procedure was followed for QC samples. In the next step, the samples were resuspendent in 100μl of water. And then centrifuged for 20 minutes in a standard centrifuge at 10,000 rpm. 95 μl of the supernatant were taken and placed in special vials suitable for the Orbitrap autosampler. The samples at this stage were ready for analysis and placed in the cooled autosampler (4 ^{o}C) and analysed in a Thermo Scientific™ Orbitrap LC/MS-MS. After the analysis of positive and negative ionization, the m/z spectrums were processed with MZmine [25]. Baseline correction was carried out as there is usually an imbalance between spectra due to the introduction of a different noise value, followed by peak detection, chromatogram deconvolution [26] and peak alignment using the random sample consensus algorithm [27]. Finally, metabolite identification was carried out using the MetaboSearch tool, where the metabolites identification based on m/z values was carried out on four bases simultaneously,, including the Human MetabolomeDataBase (HMDB), the Madison Metabolomics Consortium Database (MMCD), the Metlin database and the LipidMaps database [28].

The next step after metabolites identification is the building of a mechanistic hypothesis, i.e. putting the combination of identified metabolites in a plausible biological context. The specific work on metabolic pathway analysis was carried out using the Agilent Mass Profiler Professional (MPP). This is a powerful tool designed to accept input data, results obtained by sample analysis by LC/MS-MS, and provide high quality information about the observed relationships between samples or sample groups. It is noteworthy that in this study metabolic pathways were analyzed separately for each sample, ie at the end of the analysis 50 lists of metabolic pathways were generated based on the results obtained from the analysis of samples with LC/MS-MS.

2.2.3 Cancer risk potency

Mixtures of PCDDs/PCDFs are complex environmental mixtures of 210 interrelated chemicals composed of different dioxins and furans. For mixtures PCDDs/PCDFs, the reference chemical is 2,3,7,8 – tetrachlorodibenzo-p-dioxin

(2,3,7,8–TCDD) because it is the most toxic and best-studied of the 210 PCDDs/PCDFs. The toxicity equivalency factor (TEF) methodology was developed by the U.S. Environmental Protection Agency to evaluate the toxicity and assess the risks of a mixture of structurally related chemicals with a common mechanism of action. A TEF is an estimate of the relative toxicity of a chemical compared to a reference chemical. Toxic Equivalents, or TEQs, are used to report the toxicity-weighted masses of mixtures of PCDDs/PCDFs. The TEQ method of PCDDs/PCDFs reporting is more meaningful than simply reporting the total number of grams of a mixture of variously toxic compounds because the TEQ method offers toxicity information about the mixture. Within the TEQ method, each PCDDs/PCDFs compound is assigned a Toxic Equivalency Factor, or TEF. This factor denotes a given dioxin compound's toxicity relative to 2,3,7,8-TCDD, which is assigned the maximum toxicity designation of one. Other dioxin compounds are given equal or lower numbers, with each number roughly proportional to its toxicity relative to that of 2,3,7,8-TCDD. Developed by the World Health Organization, TEFs are used extensively by scientists and governments around the world [29], finally expressing the so-called TEQ WHO (toxicity equivalent concentration in accordance with the methodology of the World Health Organization), that uses units of grams-TEQ. The EPA uses TEQ WHO to report emissions of PCDDs/PCDFs from known sources to the open environment in its Inventory of Sources of Dioxin in the United States and similar practices have been adopted worldwide, including all the data presented in this study. To obtain the number of grams-TEQ of a dioxin mixture, one simply multiplies the mass of each compound in the mixture by its TEF and then totals them.

EPA has classified 2,3,7,8-TCDD as a Group B2, meaning a probable human carcinogen [30]. With regard to 2,3,7,8-TCDD, EPA has calculated an inhalation cancer slope factor of $1.5 \cdot 10^5$ $(mg/kg/d)^{-1}$ and an inhalation unit risk estimate of 3.3×10^{-5} $(pg/m^3)^{-1}$ for 2,3,7,8-TCDD. A similar slope factor of $1.5 \cdot 10^5$ $(mg/kg/d)^{-1}$ has been proposed for orally administered 2,3,7,8-TCDD, which correspond to an oral unit risk factor of 4.5 $(\mu g/L)^{-1}$.

3 Results

3.1 Ambient air concentrations of PCDDs/PCDFs

Analysis of ambient air samples (both particle and gaseous phase) showed that the levels of PCDDs/PCDFs in the surrounding area were 1.8 pg/m3 TEQ WHO (toxicity equivalent concentration in accordance with the methodology of the World Health Organization). These levels are significantly higher than the ones reported in previous studies, where atmospheric background concentration of a typical industrial site in the wider area of Athens was found to be equal to 0.1 pg/m3 TEQ WHO, but in the same order of magnitude to the levels of landfill fires (Figure 1).

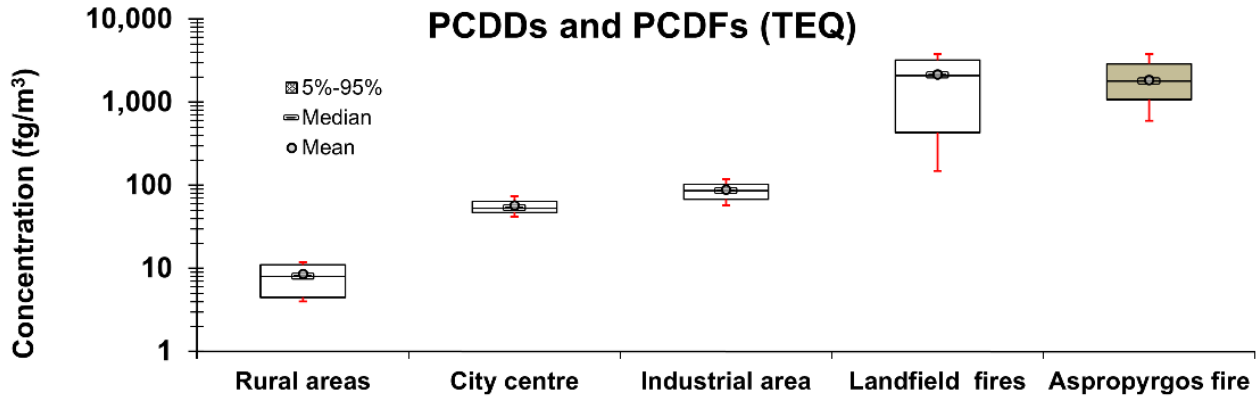

Figure 1. Levels of PCDDs/PCDFs (TEQ) at various Athens sub-areas, as well as during accidental fire events

3.2 Internal dose of PCDDs/PCDFs

To calculate the additional burden of exposure for the living population due to the accidental event, it was critical to estimate the actual background exposure to dioxins/furans. For this purpose, the data of a biomonitoring study held in Athens in 2006 [31] were used, according to which the average PCDDs/PCDFs TEQ concentration in blood serum was equal to 7.3 pg/g lipids. Exposure reconstruction using biomonitoring data provided a comprehensive overview of the actual daily uptake from all potential pathways (e.g. ambient air and food) and routes (inhalation and oral). According to the biokinetic model, this blood concentration level corresponds to an equivalent daily intake of PCDDs/PCDFs of

about 0.002 pg_TEQ/kg_bw/d. By using these uptake levels, the respective time course of the concentration of PCDDs/PCDFs in the blood for a period of 30 years is described with the blue solid line of Figure 2. As shown in Figure 2, exposure to the accidental fire fumes results in a significant increase of dioxin levels in the blood, up to 18 pg/g lipids (red thick line). Due to the high level of PCDDs/PCDFs persistence, the concentrations in the blood will remain higher than the ones of the background for several years. The elevated exposure levels in adult mothers, are very important for the developing fetus. The constantly elevated blood levels in mother, result in similar levels of PCDDs/PCDFs in the fetus blood. Considering that fetus has a higher amount of adipose tissue, thus a higher capacity of bioaccumulation of lipophilic compounds, this additional burden of PCDDs/PCDFs at that early life stage, results in continuously higher internal exposure (AUC is almost 20% higher) during the entire lifespan. Similarly, breast milk of exposed mothers is expected to have a concentration of PCDDs/PCDFs of almost 10 pg/g lipids, resulting in a significant exposure burden during the breast feeding period, which in turn results in an increase of the entire lifespan AUC of almost 30%.

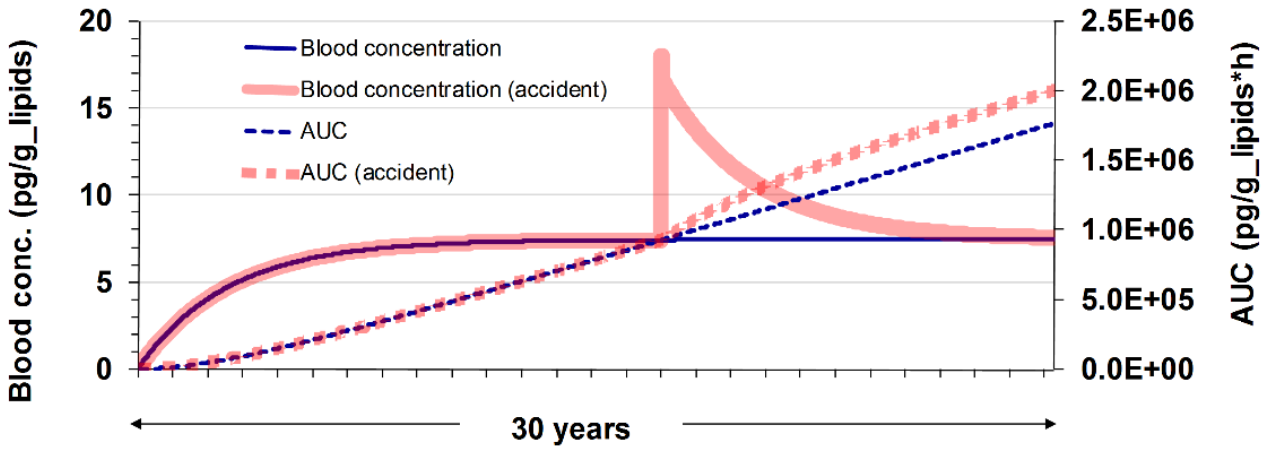

Figure 2. Internal exposure to dioxins/furans under (a) usual conditions (continuous line) and (b) under accidental release (dotted line)

3.3 Biosample analysis

It has also to be noted that both the elevated internal exposure to PCDDs/PCDFs, as well as the activation of molecular pathways related to cancer have been verified by biomonitoring of the population leaving nearby the plastic recycling plant. For this purpose, human blood was sampled from 50 individuals, including both adults and children. The results of the analysis (Figure 3) indicated that the levels of PCDDs/PCDFs were higher (~12.4 pg/g_lipid) to the ones of the background (~7.4 pg/g_lipid), indicating that the accidental event resulted in increased internal exposure, as already was predicted by the exposure assessment and the PBPK model. It is also interesting that PCDDs/PCDFs levels were higher in adults than in children, reflecting (a) the higher background of exposure to these compounds to of the older generations and (b) the continuous but slow decrease of the PCDDs/PCDFs in the environment, after POPs convention [32].

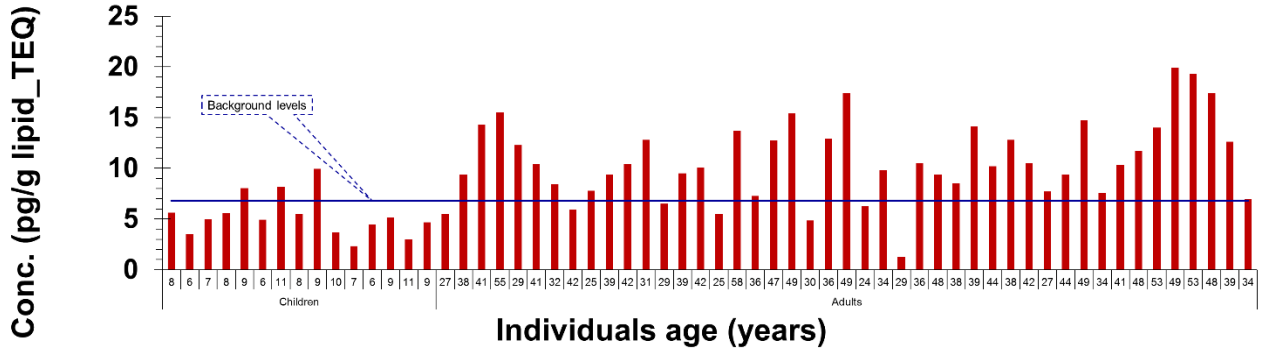

Figure 3. PCDDs/PCDFs (in TEQ levels in blood of the exposed individuals after the accidental event

In addition, untargeted metabolomics analysis indicated that increased levels of unsaturated vs saturated fatty acids were identified compared to controls (population non-exposed to the fumes). This finding could indicate perturbation of cholesterol homeostasis; the latter, is highly related to AhR deregulation. The aryl hydrocarbon receptor does not only act as a transcription factor binding the dioxin responsive element (DRE) and activating metabolizing enzymes such as CYP1A1 and CYP1B1. AhR is also involved in the regulation of other pathways in a DRE-independent manner. As an example, AhR regulates cholesterol biosynthesis by interacting with the sterol element-binding protein 2 (SREPB2) transcription factor. Furthermore, cross-talks between AhR and some nuclear receptors such as the estrogen receptor (ER) and the androgen receptor (AR) are well described [33]. AR itself influences cholesterol homeostasis by interacting with the liver X receptor (LXR), which can regulate sulfotransferases and thereby androgens activity [34]. Exposure to dioxins eventually leads to alterations in several metabolic pathways, including hepatic lipogenesis, perturbed TCA cycle, disrupted carbohydrate and amino acid metabolism as well as inhibition of de novo fatty acid biosynthesis. The results of this analysis, indicated the activation of pathways associated to cancer, thus verifying the hypothesis of increased cancer risk due to exposure to dioxines and furans. Additional identified pathways related to cancer included sphingosine and sphingosine-1-phosphate metabolism pathways. Sphingolipid metabolites, ceramide, sphingosine, and sphingosine 1-phosphate, have emerged as a new class of lipid biomodulators of various cell functions. These functions include adhesion, cellular growth and differentiation, apoptosis and surface antigens. An increase in the concentration of glycosphingolipids on the cellular membrane of cancer cells leads to significant changes of the antigenic properties, including the formation of tumor-associated carbohydrate antigens, and a loss of adhesion, as well as increased motility enhancing invasion and metastasis. As a result, altered sphingolipid metabolism contributes to cancer progression [35].

3.4 Estimation of cancer risk

This additional internal exposure burden which is also illustrated as the difference of the area under the curve (AUC) between the blue and the red dotted lines, will result in an increased risk of cancer associated with exposure to PCDDs/PCDFs. The obtained AUC, was translated into a new equivalent chronic uptake dose, equal to 0.00226 pg_TEQ/kg_bw/d. Uptake estimates were translated into cancer risk estimates, using the slope factor proposed by the US Environmental Protection Agency (1985, 1997).

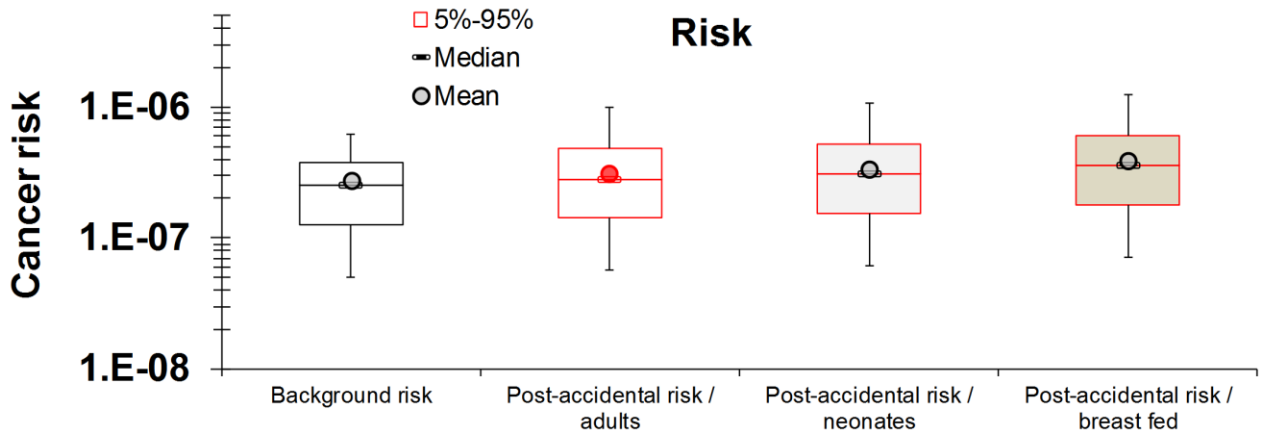

Figure 4. Estimated cancer risk for the exposed population before and after the accidental fire

Accounting for variability in exposure estimates, fat and blood lipids content, distributions of exposure estimates were derived. Based on the background level of exposure to PCDDs/PCDFs of the general population the risk of chronic exposure was estimated (mean value) equal to $2.57 \cdot 10^{-7}$. For the population exposed over 6 days to the PCDDs/PCDFs fumes emitted from the recycling plant fire the respective risk (mean value) was up to $2.91 \cdot 10^{-7}$, indicating an increase of 13% in the 30-year cancer risk. It has to be noted that for the upper bound of the post-accidental risk estimates are close to 10^{-6} (Figure 4**Error! Reference source not found.**).

The respective risk is expected to be higher for neonates, whose mothers were exposed to the fumes of the accidental fire during pregnancy. The higher levels of PCDDs/PCDFs in the blood during pregnancy will result in continuously higher levels during their entire life span, resulting in an increase of the estimated lifetime risk of almost 20%. The estimated lifetime risk is expected to be even higher for neonates that are also breast fed; breast feeding is considered a major source of lifetime exposure to dioxins and the increased levels of exposure to PCDDs/PCDFs are considered to contribute to up to 32%.

4 Discussion

The current study dealt up with the assessment of long-term effects of exposure to PCDDs/PCDFs due to an accidental event occurred in a plastic recycling plant. Translating short term environmental contamination events into long term exposure and effects comprise a major scientific challenge. Aiming at addressing this issue, a comprehensive methodological framework was applied that integrates both environmental, exposure, internal dosimetry and biomonitoring data, namely INTEGRA [14]. Considering that the exposure burden due to the accidental event is not relevant to a long term increase of external intake, by simply using a daily intake rate and a slope factor, would not allow as to properly account for the actual risk, resulting to significant underestimation, since the actual increase in the intake, would be accounted only for the 6 days of the fire. On the contrary, the use of internal dosimetry metrics, allowed us to track the AUC, which is a more realistic metric for associating exposure and effect. In addition, inter-individual variability of the population has been taken into account in terms of age, body fat, lipids in the blood and smoking status. As a result, due to the accidental event, an increase of the long term cancer risk associated to PCDDs/PCDFs of 13% for the adult local population was estimated, while significantly elevated risk estimates were calculated for neonates (20% increased risk) who's their mothers were exposed during pregnancy and for breast fed infants (32% increased risk). The fact that the increase of the associated risk is in the range of 10^{-7}, indicates that the estimated risk is not negligible and is within the range of the background risk associated to PCDDs/PCDFs exposure from multiple sources. On the other hand, considering the continuous efforts for reducing the emissions of PCDDs/PCDFs in the environment and the continuously reducing levels globally identified in human matrices and especially analysis of human breast milk that is a non-invasive method, the additional burden of such type of accidental events will be more and more important. We need to take also into account that exposure to the specific event might be higher for part of the population (living closer to the center of the plume) and also that there are significant differences in the susceptibility of the population against carcinogenic agents. In this case the estimated risks could be higher (upper part of the risk estimates distribution), in the order of magnitude of 10^{-6}; the latter is considered (unofficially) as the acceptable risk for environmental factors. Moreover, the effect on the most vulnerable population (fetus and breast

feeding neonates) are expected to be significantly higher than the adults. This is the result of the major toxicokinetic characteristics of PCDDs/PCDFs; prenatal and postnatal exposure comprise a significant contributor of actual internal exposure to dioxins, providing the heritage of PCDDs/PCDFs initial burden of exposure. It is also important that these results were also verified by extensive analysis of human biosamples collected after the accidents; post accidental biomonitored PCDDs/PCDFs levels were higher than the ones of the controls, while pathway analysis revealed the perturbation of cancer-related pathways (AhR and sphingosine and sphingosine-1-phosphate metabolism). This case provides additional evidence that the different elements of the connectivity paradigm can be functionally integrated among them to provide a coherent framework towards the development of adverse outcome pathways [36,37].

5 Conclusion

Although waste recycling is considered one of the most environment friendly and sustainable waste management option, accidental events might result in significant contamination of the surrounding area, affecting adversely the population leaving nearby. The study herein, applied a comprehensive methodological framework for addressing one of the major problems related to accidental events and the release of bioaccumulative, persistent and toxic compounds, which is the translation of a short-term exposure event into a long-term exposure estimate. Towards this aim, the INTEGRA computational platform allowed the assimilation of different type of both environmental and HBM data, for estimating the chronic internal exposure to PCDDs/PCDFs. For estimating the associating risks, the concept of TEQ for the mixture of PCDDs/PCDFs was employed, using as a TEF reference compound 2,3,7,8-TCDD, which has a well-established slope factor. Based on the above, an average increase of 13% of cancer risk attributed to PCDDs/PCDFs was estimated population. A key finding of the study was that neonates and breast fed infants face even higher lifetime risks, and also that these risks were quantified (20% and 32% respectively). The perturbation of pathways related to PCDDs/PCDFs mediated cancer, were also verified by metabolomics and pathways analysis. This cancer risk estimates are going to be re-evaluated after the analysis of environmental media other than air (soil and water of the surrounding area) and the affected food web, to quantify the potential contribution of additional exposure pathways.

6 REFERENCES

1. Farmer, T.D., Shaw, P.J., Williams, I.D.: Destined for indecision? A critical analysis of waste management practices in England from 1996 to 2013. Waste management (New York, N.Y.) **39**, 266-276 (2015). doi:10.1016/j.wasman.2015.02.023

2. Cole, P., Trichopoulos, D., Pastides, H., Starr, T., Mandel, J.S.: Dioxin and cancer: a critical review. Regulatory toxicology and pharmacology : RTP **38**(3), 378-388 (2003).

3. Lucier, G.W., Portier, C.J., Gallo, M.A.: Receptor mechanisms and dose-response models for the effects of dioxins. Environ Health Perspect **101**(1), 36-44 (1993).

4. Dong, Z., Yuan, G., Hu, J.: Uncertainty analysis in 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) cancer dose-response for three occupational cohorts. Environ Int **88**, 53-59 (2016). doi:10.1016/j.envint.2015.12.010

5. Kerger, B.D., Leung, H.-W., Scott, P., Paustenbach, D.J., Needham, L.L., Patterson, D.G., Gerthoux, P.M., Mocarelli, P.: Age- and Concentration-Dependent Elimination Half-Life of 2,3,7,8-Tetrachlorodibenzo-p-dioxin in Seveso Children. Environmental Health Perspectives **114**(10), 1596-1602 (2006). doi:10.1289/ehp.8884

6. Milbrath, M.O., Wenger, Y., Chang, C.W., Emond, C., Garabrant, D., Gillespie, B.W., Jolliet, O.: Apparent half-lives of dioxins, furans, and polychlorinated biphenyls as a function of age, body fat, smoking status, and breast-feeding. Environ Health Perspect **117**(3), 417-425 (2009). doi:10.1289/ehp.11781

7. Michalek, J.E., Tripathi, R.C.: Pharmacokinetics of TCDD in veterans of Operation Ranch Hand: 15-year follow-up. Journal of toxicology and environmental health. Part A **57**(6), 369-378 (1999).

8. Emond, C., DeVito, M., Warner, M., Eskenazi, B., Mocarelli, P., Birnbaum, L.S.: An assessment of dioxin exposure across gestation and lactation using a PBPK model and new data from Seveso. Environ Int **92-93**, 23-32 (2016). doi:10.1016/j.envint.2016.03.015

9. Jeanneret, F., Boccard, J., Badoud, F., Sorg, O., Tonoli, D., Pelclova, D., Vlckova, S., Rutledge, D.N., Samer, C.F., Hochstrasser, D., Saurat, J.-H., Rudaz, S.: Human urinary biomarkers of dioxin exposure: Analysis by metabolomics

and biologically driven data dimensionality reduction. Toxicology Letters **230**(2), 234-243 (2014). doi:https://doi.org/10.1016/j.toxlet.2013.10.031

10. Matsubara, T., Tanaka, N., Krausz, K.W., Manna, S.K., Kang, D.W., Anderson, E.R., Luecke, H., Patterson, A.D., Shah, Y.M., Gonzalez, F.J.: Metabolomics identifies an inflammatory cascade involved in dioxin- and diet-induced steatohepatitis. Cell Metab **16**(5), 634-644 (2012). doi:10.1016/j.cmet.2012.10.006

11. Forgacs, A.L., Kent, M.N., Makley, M.K., Mets, B., DelRaso, N., Jahns, G.L., Burgoon, L.D., Zacharewski, T.R., Reo, N.V.: Comparative metabolomic and genomic analyses of TCDD-elicited metabolic disruption in mouse and rat liver. Toxicol Sci **125**(1), 41-55 (2012). doi:10.1093/toxsci/kfr262

12. Landi, M.T., Bertazzi, P.A., Baccarelli, A., Consonni, D., Masten, S., Lucier, G., Mocarelli, P., Needham, L., Caporaso, N., Grassman, J.: TCDD-mediated alterations in the AhR-dependent pathway in Seveso, Italy, 20 years after the accident. Carcinogenesis **24**(4), 673-680 (2003).

13. Fernando, S., Jobst, K.J., Taguchi, V.Y., Helm, P.A., Reiner, E.J., McCarry, B.E.: Identification of the halogenated compounds resulting from the 1997 Plastimet Inc. fire in Hamilton, Ontario, using comprehensive two-dimensional gas chromatography and (ultra)high resolution mass spectrometry. Environ Sci Technol **48**(18), 10656-10663 (2014). doi:10.1021/es503428j

14. Sarigiannis, D., Karakitsios, S., Gotti, A., Handakas, E., Papadaki, K.: INTEGRA: Advancing risk assessment using internal dosimetry metrics. Toxicology Letters **238**(2), S110-S111 (2015).

15. de Winter-Sorkina, R., Cassee, F.R.: From concentration to dose: factors influencing airborne particulate matter deposition in humans and rats. In. RIVM National Institute for Public Health and the Environment, Bilthoven, (2002)

16. Mortensen, J.D.: A Study of Age Specific Human Respiratory Morphometry: Final Report. UBTL Division, University of Utah Research Institute, (1983a)

17. Mortensen, J.D., et al.: Age related morphometric analysis of human lung casts. Extrapolation of Dosimetric Relationships for Inhaled Particles and Gases. Academic Press., San Diego, CA. (1988)

18. Mortensen, J.D., Young, J.D., Stout, L., Stout, A., Bagley, B., Schaap, R.N.: A numerical identification system for airways in the lung. Anatomical Record **206**(1), 103-114 (1983b).

19. Sarigiannis, D., Papadaki, K., Kontoroupis, P., Karakitsios, S.: Advanced QSAR models for use in toxicokinetic modelling. Toxicology Letters **238**(2), S166-S167 (2015).

20. Beaudouin, R., Micallef, S., Brochot, C.: A stochastic whole-body physiologically based pharmacokinetic model to assess the impact of inter-individual variability on tissue dosimetry over the human lifespan. Regulatory Toxicology and Pharmacology **57**(1), 103-116 (2010).

21. Lee, S.K., Ou, Y.C., Andersen, M.E., Yang, R.S.H.: A physiologically based pharmacokinetic model for lactational transfer of PCB 153 with or without PCB 126 in mice. Archives of Toxicology **81**(2), 101-111 (2007).

22. Verner, M.A., Charbonneau, M., Lopez-Carrillo, L., Haddad, S.: Physiologically based pharmacokinetic modeling of persistent organic pollutants for lifetime exposure assessment: A new tool in breast cancer epidemiologic studies. Environmental Health Perspectives **116**(7), 886-892 (2008).

23. Sarigiannis, D.A., Karakitsios, S.P., Handakas, E., Simou, K., Solomou, E., Gotti, A.: Integrated exposure and risk characterization of bisphenol-A in Europe. Food and Chemical Toxicology **98**, 134-147 (2016). doi:10.1016/j.fct.2016.10.017

24. Byrd, D.M., 3rd, Allen, D.O., Beamer, R.L., Besch, H.R., Jr., Bylund, D.B., Doull, J., Fleming, W.W., Fries, A., Guengerich, F.P., Hornbrook, R., Lasagna, L., Lum, B.K., Michaelis, E.K., Morgan, E.T., Poland, A., Rozman, K.K., Smith, J.B., Swanson, H.I., Waddell, W., Wilson, J.D.: The dose-response model for dioxin. Risk analysis : an official publication of the Society for Risk Analysis **18**(1), 1-2 (1998).

25. Katajamaa, M., Miettinen, J., Orešič, M.: MZmine: toolbox for processing and visualization of mass spectrometry based molecular profile data. Bioinformatics **22**(5), 634-636 (2006).

26. Alonso, A., Marsal, S., Julia, A.: Analytical methods in untargeted metabolomics: state of the art in 2015. Frontiers in bioengineering and biotechnology **3**, 23 (2015). doi:10.3389/fbioe.2015.00023

27. Zhao, S., Yu, G.: A new image registration algorithm using SDTR. Neurocomputing **234**, 174-184 (2017). doi:10.1016/j.neucom.2016.12.055

28. Zhou, B., Wang, J., Ressom, H.W.: MetaboSearch: Tool for Mass-Based Metabolite Identification Using Multiple Databases. PloS one **7**(6), e40096 (2012). doi:10.1371/journal.pone.0040096

29. Van den Berg, M., Birnbaum, L., Bosveld, A.T., Brunstrom, B., Cook, P., Feeley, M., Giesy, J.P., Hanberg, A., Hasegawa, R., Kennedy, S.W., Kubiak, T., Larsen, J.C., van Leeuwen, F.X., Liem, A.K., Nolt, C., Peterson, R.E., Poellinger, L., Safe, S., Schrenk, D., Tillitt, D., Tysklind, M., Younes, M., Waern, F., Zacharewski, T.: Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environ Health Perspect **106**(12), 775-792 (1998).

30. USEPA: U.S. Environmental Protection Agency. Health Assessment Document for Polychlorinated Dibenzo-p-Dioxin. Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment, Office of Research and Development, Cincinnati, OH. EPA 600/8-84-014F. 1985. In. (1985)

31. Costopoulou, D., Vassiliadou, I., Papadopoulos, A., Makropoulos, V., Leondiadis, L.: Levels of dioxins, furans and PCBs in human serum and milk of people living in Greece. Chemosphere **65**(9), 1462-1469 (2006). doi:http://dx.doi.org/10.1016/j.chemosphere.2006.04.034

32. WHO: Human biomonitoring: facts and figures. In. Copenhagen: WHO Regional Office for Europe, (2015)

33. Beischlag, T.V., Morales, J.L., Hollingshead, B.D., Perdew, G.H.: The Aryl Hydrocarbon Receptor Complex and the Control of Gene Expression. Critical reviews in eukaryotic gene expression **18**(3), 207-250 (2008).

34. Jeanneret, F., Boccard, J., Badoud, F., Sorg, O., Tonoli, D., Pelclova, D., Vlckova, S., Rutledge, D.N., Samer, C.F., Hochstrasser, D., Saurat, J.H., Rudaz, S.: Human urinary biomarkers of dioxin exposure: analysis by metabolomics and biologically driven data dimensionality reduction. Toxicol Lett **230**(2), 234-243 (2014). doi:10.1016/j.toxlet.2013.10.031

35. Ryland, L.K., Fox, T.E., Liu, X., Loughran, T.P., Kester, M.: Dysregulation of sphingolipid metabolism in cancer. Cancer biology & therapy **11**(2), 138-149 (2011).

36. Gutsell, S., Russell, P.: The role of chemistry in developing understanding of adverse outcome pathways and their application in risk assessment. Toxicology Research **2**(5), 299-307 (2013).

37. Ankley, G.T., Bennett, R.S., Erickson, R.J., Hoff, D.J., Hornung, M.W., Johnson, R.D., Mount, D.R., Nichols, J.W., Russom, C.L., Schmieder, P.K., Serrrano, J.A., Tietge, J.E., Villeneuve, D.L.: Adverse outcome pathways: A conceptual framework to support ecotoxicology research and risk assessment. Environmental Toxicology and Chemistry **29**(3), 730-741 (2010).